(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) Publication number : **0 629 350 A1**

(12) EUROPEAN PATENT APPLICATION

(21) Application number : **94810352.8**

(51) Int. Cl.[5] : **A23J 3/34**, A23L 1/305

(22) Date of filing : **14.06.94**

(30) Priority : **16.06.93 GB 9312369**

(43) Date of publication of application :
21.12.94 Bulletin 94/51

(84) Designated Contracting States :
AT BE CH DE DK ES FR GB GR IE IT LI LU NL PT SE

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(54) Milk protein hydrolysates.

(57) The use of cow's milk protein hydrolysate substantially free of allergenic proteins to induce cow's milk protein tolerance in children susceptible to cow's milk allergy and in the prophylaxis or treatment of type 1 diabetes mellitus in children susceptible to such disease.

Figure 1

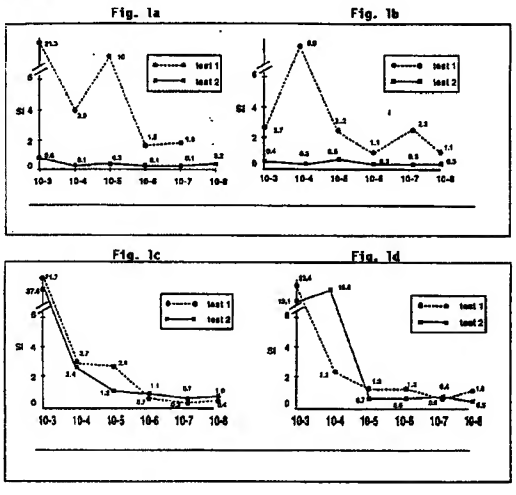

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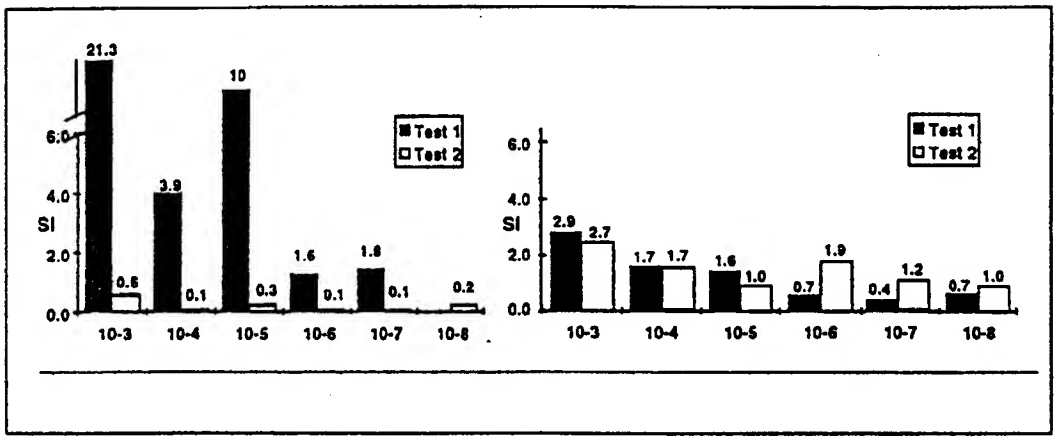
Figure 2

Fig. 2a

Fig. 2b



The invention relates to milk protein hydrolysates and their use to induce immunologic tolerance in infants suffering from cow's milk allergy.

EPA 421 309 (Sandoz Nutrition Ltd.) discloses non-allergenic cow milk protein hydrolysates having a high degree of hydrolysis whilst having a low content of free amino acids. The non-allergenic milk protein hydrolysates disclosed in EPA 421 309 are indicated for use as protein supply replacement in infants having intolerance against dietary proteins such as cow's milk proteins.

The terms non-allergenic hydrolysates and hydrolysates substantially free of allergenic proteins as used herein are interchangeable. They refer to protein hydrolysates that can be administered to infants having intolerance against dietary proteins, more particularly cow's milk proteins, without inducing allergic reactions.

In allergic disease, the type of allergy is related with the age to the child as food allergy mostly occur in infants. Cow's milk allergy is a common food allergy in infants, the disease prevalence ranging from 2 to 7,5 % of all children. Various symptoms can occur. Symptoms can be immediate (e.g. anaphylaxis and urticaria) which occur in half of the patients or less specific (e.g. vomiting or diarrhoea) which represent delayed clinical reactions in the other half of patients. Diagnostic procedure is well standardized with the criteria given by the European Society for Paediatric Gastroenterology and Nutrition Working Group (ESPGAN J. Pediatr. Gastroenterol. Nutr. 1992; 14: 108-12). Complete exclusion of cow's milk proteins and diet with hydrolysed formulas represent the treatment of choice of this condition. Cow's milk allergy disappears spontaneously. Cow's milk tolerance tends to appear at 2 to 4 years of age, whereby it is been suggested for safety reasons to avoid administration of milk proteins to children suffering from cow's milk allergy before they are 6 to 7 years old.

It has now surprisingly been found that non-allergenic whey protein hydrolysates are capable of inducing cow's milk protein tolerance such that cow's milk allergy disappears substantially faster than normally would be expected for spontaneously (i.e. naturally) acquired tolerance.

The invention accordingly provides a method of inducing cow's milk protein tolerance in children susceptible to cow's milk allergy by administering to a child in need of such treatment a whey protein hydrolysate substantially free of allergenic proteins.

The whey protein hydrolysate free of allergenic proteins is conveniently administered in the form of a complete formula diet.

Another embodiment of the invention is the use of whey protein hydrolysate substantially free of allergenic proteins for the manufacture of a dietary composition for the induction of cow's milk protein tolerance.

Examples of non-allergenic whey protein hydrolysates suitable for use in the method of the invention are hydrolysates obtainable from whey proteins enzymatically hydrolysed by trypsin-chymotrypsin in the presence of a cationic serine endoprotease of the elastase 2 type, e.g. at a temperature of from 35°C to 50°C and a pH between 7.0 and 9.0

The whey protein fraction employed as starting material will preferably be substantially free of macrolipids; i.a. to facilitate enzymatic hydrolysis.

The whey proteins may have been pretreated, e.g. subjected to microfiltration/ultrafiltration, to remove most of large proteins having a molecular weight of more than 60'000 Daltons. Such pretreated whey protein mixture is hereinafter designated "selected whey protein". It will still contain some bovine serum albumine (BSA) having a molecular weight of ca. 66.000 Da.

The use of elastase 2 allows the elimination of allergenic epitopes and provides a product having a high effective degree of hydrolysis (19 % or more), a low content of free amino acids while having a high content of di- to decapeptides and in particular of tetra- to decapeptides.

Preferably the whey protein, more preferably the selected whey protein, is initially subjected to a gastric phase prehydrolysis, e.g. by heating a solution of (selected) whey protein in water to 43 ± 4°C and treating said solution with pepsin at pH between 2.0 and 3.0.

Pepsin hydrolysis of BSA results for example in very little small peptides but facilitates by denaturation the subsequent elimination of peptide fragments of high molecular weight (30.000 to 40.000 Da) by treatment with other enzymes. Elastase 2 allows i.a. the reduction of peptides having an intermediate size (ca. 10.000 Da) and induces a shift of the peptide profile in favour of low molecular weight peptides.

Accordingly, the non-allergenic whey protein hydrolysates particularly suitable for use in the method of the invention are obtainable by physiological hydrolysis of selected whey protein. Said physiological hydrolysis involves a gastric phase, i.e. a pepsin prehydrolysis at pH between 2.0 and 3.0, followed by an enzymatic treatment of the prehydrolysate with a mixture of trypsin-chymotrypsin and a cationic serine endoprotease type elastase 2. Suitable conditions for such physiological hydrolysis are disclosed in EPA 421 309.

The effective degree of hydrolysis of non-allergenic protein hydrolysates obtainable by physiological hydrolysis will conveniently be in the range of from 20 % to 32 %. The free amino acid content of whey protein hydrolysates suitable for use in the method of the invention, is preferably below 15 %, more preferably in the range of from 1 to 13 % by weight.

The effective degree of hydrolysis is determined according to the formula:

$$\frac{\text{g of N in free } NH_2 \text{ groups}}{\text{g of total N } (NH, NH_2)} \times 100$$

A typical molecular weight distribution of non-allergenic whey protein hydrolysates obtainable by physiological hydrolysis suitable for use in the method of this invention, is as follows (determined by UV detection at 215 nm);

	% by weight from	more preferably	e.g.
MW > 5000	0.5 to 4.0	2.8 to 3.8	3.3
5000 > MW > 3000	2 to 10	5 to 10	5.2
3000 > MW > 1000	29 to 36	33.5 to 35.5	33.9
1000 > MW > 500	26 to 31	29 to 31	30.9
500 > MW > 300	14.6 to 29.6	15 to 21	20.6
300 > MW	5.9 to 12.9	5.7 to 7.7	6.1

Such non-allergenic whey protein hydrolysates are preferably administered in admixture with non-allergenic casein hydrolysates in a weight ratio whey protein hydrolysate: casein protein hydrolysate of 50:50 to 70:30, preferably 60:40, such that its composition in g amino acid per 100 g hydrolysate is similar to that of mother's milk.

Non-allergenic casein protein hydrolysates are obtainable by enzymatic treatment of casein with a mixture of trypsin-chymotrypsin and a cationic serine endoprotease type elastase 2. Casein hydrolysates are in general less problematic than whey protein hydrolysates from the allergenicity point of view. Thus it is in general not necessary to subject casein - in addition to the aforementioned enzymatic treatment - to a gastric phase hydrolysis.

Typically , the casein used as starting material for preparation of casein hydrolysates suitable for use in the method of the invention, will be casein substantially free of glycoprotein fraction; the casein employed is conveniently rennet casein, i.e. as obtained by coagulation with pepsin, e.g. bovine pepsin.

A typical molecular weight distribution of non-allergenic casein hydrolysates suitable for use in admixture with non-allergenic whey protein hydrolysates in the method of the invention is as follows (determined by UV detection at 215 nm):

	% by weight range	e.g.
MW > 5000	0 to 1	0
5000 > MW > 3000	0 to 1	0.1
3000 > MW > 1000	17 to 26	18.6
1000 > MW > 500	33 to 40	37.4
500 > MW > 300	26 to 31	29.2
300 > MW	11 to 16	14.7

The effective degree of hydrolysis of particularly suitable casein hydrolysates lies in the range of from 20 to 30 %.

The free amino acid content in the casein hydrolysates suitable for use in the invention is preferably below 15 % and more preferably in the range of from 8 to 13 %.

The whey protein hydrolysates and casein protein hydrolysates may be treated with a proteolytic enzyme from the fungus Aspergillus oryzae or with a pancreatic proteinase extract for taste improvement. Such enzymes are commercially available. Typical examples thereof include COROLASE 7092 and COROLASE PP (Röhm).

The treatment of milk proteins with a COROLASE 7092 type of proteolytic enzyme is conveniently at a pH of about 8 and employing ca. 1.0 to 1.5 % enzyme/protein substrate having an activity of about 2000 m

UHb/mg enzyme (see Example 4 hereinafter) during ca. 1¼ to 1¾ hours.

The proteolytic enzyme from Aspergillus oryzae is conveniently added to the protein mixture comprising an effective amount of pancreatic enzymes (trypsin-chymotrypsin and optionally elastase 2), preferably after 1 hr to 1½ hr treatment with pancreatic enzymes at 41 to 47° C.

Treatment with 1.0 to 1.5 % COROLASE PP type enzyme/protein substrate at a pH of about 8 during 1¼ to 1¾ hours at 41 to 47° C allows similar taste improvement.

The preparation of the starting materials, the hydrolysis conditions and working up of the hydrolysates including any pasteurization, ultrafiltration, concentration and drying steps may be carried out in a manner known per se, e.g. analogous to the methods disclosed in EPA 421 309 or as exemplified hereinafter.

It will be appreciated that the composition of the hydrolysates obtained by hydrolysis of milk proteins or milk protein fractions will depend on the hydrolysis conditions.

Preferably the conditions will be selected such that the hydrolysate has an effective degree of hydrolysis and a free amino acid content within the ranges specified herein.

In general it will be preferred to use a non-allergenic whey protein hydrolysate, more preferably a whey protein hydrolysate: casein hydrolysate mixture in a weight ratio in the range of from 5:1 to 1:1, most preferably 1.5:1.

The whey protein hydrolysate fraction to be employed comprises conveniently less than 35 % by weight preferably from 20 to 33 % by weight of peptides containing 2 to 5 amino acids and/or from 35 to 60 % by weight of peptides having 4 to 10 amino acids when determined by UV detection at 215 nm. (It will be appreciated that the values will vary within said ranges to some extent due to natural variation of the composition of the starting material, but also depending on the detection method employed: the chromatographic analysis by UV 215 nm detection and by Refraction Index detection will give different results).

The ability of milk protein hydrolysates to induce cow's milk protein tolerance is indicated by results obtained employing the Lymphocyte stimulation test (LST).

Background

Lymphocyte stimulation test (LST) was performed with sterile blood of a cow's milk allergic child in order to study cellular response to the offending proteins before, and after 3 months exclusion diet. The child developed during the treatment with DAMIRA® (Sandoz Nutrition Ltd.) a cellular tolerance showed by cellular stimulation only be higher concentration of the offending proteins.

The mild protein hydrolysate used as test mixture is a commercially available product, designated DAMIRA® (Sandoz Nutriton Ltd.).

DAMIRA® comprises a hydrolysate from 60 % whey protein and 40 % rennet casein. The whey protein hydrolysate has been obtained by physiological hydrolysis of whey protein, substantially free of macrolipids and lactose, i.e. involving a gastric phase (pepsin hydrolysis) and a pancreatic hydrolysis (with trypsin/chymotrypsin and elastase 2), essentially as disclosed in Example 1 hereinafter. The rennet casein hydrolysate has been obtained essentially as disclosed in Example 7 hereinafter.The DAMIRA® composition is given in Example 8.

Test Description

Lymphocyte stimulation test

This test is performed on the lymphomonocyte fraction isolated from sterile heparinized blood after centrifugation on Ficoll (Pharmacia). The cells are suspended on a buffer solution (TC 199 Hanks), centrifugated and washed 3 times. They are then added to a solution of AB human serum (at a concentration of 20 %) with culture medium (RPMI 1640). 10^6 cells/ml are incubated in quaduplicates in 96 well plates with the antigens at the different concentrations. Six different logarithmic concentrations (from 10^{-8} to 10^{-3} mg/ml of whole milk, casein, α-lactalbumin, β-lactoglobulin and a milk protein hydrolysate product are studied. After incubation for 7 days, the cell DNA is labelled with methyl-H3 thymidine; collected on a paper filter and mixed with a scintillation liquid. Radioactivity expressing the cell proliferation is measured on a Beta counter. The results are expressed either as total counts per minute (cpm) or as stimulation index (SI):

$$\frac{\text{cpm with antigen}}{\text{cpm in negative controls}}.$$

An index > 3 is interpreted as positive. Negative controls without antigen; phytohemagglutinin and tetanus toxoid are used as positive controls.

Results

Figure 1 represents the cellular response to whole cow milk and the 3 major antigens at diagnosis (test 1) and after 3 months (test 2) treatment with DAMIRA®. Fig. 1a gives the Stimulation Index (SI) for whole milk concentrations, Fig. 1b for α-lactalbumin concentrations. Fig. 1c for β-lactoglobulin concentrations and Fig. 1d for casein concentrations.

Stimulation indexes are higher for whole cow milk at all the concentrations at the time of test 1 as compared to test 2.This tendency is also observed for alpha-lactalbumin and β-lactoglobulin. The index was positive until 10-5 mg/ml at test 1 and negative at all concentrations at test 2 for whole cow milk. It was negative at 10-4 mg/ml for β-lactoglobulin at both tests; positive at 10-4 at test 1 and negative for all concentrations at test 2 for α-lactoalbumin and for casein positive at 10-4 at test 2 and negative at the same concentration at test 1.

Figure 2 compares whole cow milk (Fig. 2a) and DAMIRA® (Fib. 2b) at 3 months interval. Stimulation indexes were positive up to 10-5 mg/ml for milk at test 1 whereas stimulation indexes for DAMIRA® were negative at all the concentrations at test 1. At test 2, stimulation indexes were negative at all concentrations for both. In contrast, lymphocyte proliferation was less with whole cow milk than with DAMIRA® at all concentrations tested. These results suggest the apparition of an immunologic tolerance during the diet.

The positive results with DAMIRA® are confirmed in further tests.

In view of its tolerance inducing effect, the protein hydrolysates are also indicated for prophylaxis and therapy of infants and children having a genetic predisposition to type I diabetes mellitus:

It has indeed been suggested that Bovine Serum Albumin (BSA) triggers the immune system. Anti-BSA antibodies, in particular IgG anti-BSA antibodies bind to the endogenous antigen p69 which is located on the surface of pancreatic β-cells and has a chemical structure similar to the portion of the BSA peptide triggering in allergic reaction. Such immune attack reduces the β-cell population leading to the development of type I diabetes millitus.

Administration of the milk protein hydrolysate disclosed herein prevents destruction of pancreatic beta cells in susceptible persons. Timely diagnosis of genetic predisposition to type I diabetes mellitus will accordingly allow prophylaxis and therapy of type I diabetes mellitus in susceptible infants and children by treatment with protein hydrolysates. The treatment will conveniently be continued till the children acquired cow milk tolerance.

For use in the methods of the invention, the protein hydrolysates according to the invention are conveniently administered in nutritionally acceptable composition form. Such compositions may comprise carbohydrate and fatty acid sources, vitamins, minerals and trace elements.

Said compositions are preferably in the form of a complete formula diet (in liquid or powder form), such that, when used as sole nutrition source essentially all daily caloric, nitrogen, fatty acid, vitamin, mineral and trace element requirements are met.

For infants, the daily caloric amount to be supplied will in general lie in the range of from 100 to 180 Kcal per kg body weight. The contribution of the nitrogen source (i.e. the whey/casein hydrolysate of the invention), carbohydrate source and lipid source to the total daily amount may vary within wide ranges. In typical compositions of the invention, the carbohydrate source provides for 45 to 68 %, the fatty acid sources for 25 to 50 % and the protein hydrolysate of the invention for 7 to 15 % of the total energy supply of the composition.

An example of carbohydrates particularly suitable for use in the complete diet for infants includes a mixture on the basis of maltodextrines (10 to 25 %) and lactose (90 to 75 %), unless the infant requires a diet having a low lactose content, in which case the carbohydrate source will conveniently be quasi exempt of lactose (< 1 % lactose).

Examples of suitable fatty acid sources include triglyceride oils and phospholipids.

The carbohydrates employed for composition for adults are preferably primarily a mixture on the basis of maltodextrines having a low mono- and disaccharide content (< 5 % by weight of the total carbohydrate content), a very low content of alimentary fibers and being quasi exempt of lactose. Preferably such compositions will have a total lactose content of less than 1 % by weight of the protein hydrolysate present in the formulation.

Examples of vitamins suitable for incorporation in the composition of the invention include vitamin A, vitamin D_3, vitamin E, vitamin k_1, vitamin C, folic acid, thiamin, riboflavin, vitamin B_6, vitamin B_{12}, niacinamid, biotin, l-carnitine, choline and panthotenic acid in physiologically acceptable form. Depending on the contemplated use, the incorporation of taurine and/or hypotaurine, of L-cystine, resp. supplementation of threonine, may be useful. The amount of amino acids added to the composition of the invention, in free amino acid form or in small peptide form, will preferably be such that it does not substantially influence the composition of the non-allergenic hydrolysates employed in the method of the invention e.g. in terms of free amino acid content, effective degree of hydrolysis, content of peptides having 4 to 10 amino acids and the like.

Examples of minerals and trace elements suitable for incorporation in the composition of the invention include sodium, potassium, calcium, phosphorous, magnesium, manganese, copper, zinc, iron, selenium, chro-

mium and molybdenum in physiologically acceptable form.

It will be appreciated that the minimum daily requirements of vitamins, minerals and trace elements will depend on the person to be treated. In general, the daily minimum requirements are determined by governmental authorities; they may accordingly vary from country to country.

In the following Examples, which illustrate the invention, % and parts are by weight unless stated otherwise and temperatures in centigrades.

EXAMPLE 1 - Selected whey protein hydrolysate

1.1 Preparation of Protein Solution

In a tank of 12'000 are introduced 7500 l demineralised water at a temperature of 25 ± 2°C.

900 kg of delipidated delactosed lactoserum are gradually dissolved therein. The mixture is rinsed with 600 l of demineralised water having a temperature of 25 ± 2° and the volume adjusted to 11 500 l.

The hydration time is determined at 1 hour.

1.2 Thermic Treatment

The pH of the solution is then adjusted at 4.6 ± 0.1 employing a prepared mixture of citric acid and lactic acid (Mixture A; see below, Ex. 2.1).

The mixture is pasteurized, at pH 4.6 by flash heat treatment at 80° C during 1 minute.

The temperature of the mixture is then increased to 43 ± 2° C and the pH adjusted to pH 2.6 ± 0.1 employing a 33 % hydrochloric acid solution.

1.3 Pepsine hydrolysis

0.2 g bovine pepsine (BOVIPEP; Lab. Presure Granday) / kg protein are then added and the reaction vessel shuttled during 1 hour while maintaining the temperature at 43°C.

1.4 Demineralisation by Ultrafiltration

The pH is adjusted to pH 8 ± 0.1 employing a preprepared alkaline mixture B (see below). The temperature is maintained at 43 ± 2° C.

The mixture is ultrafiltrated (on IRIS 3038 membranes - Rhône Poulenc; membrane surface 80 m^2, temperature 50°C) till obtention of 2500 l of permeate. At this stage, the diafiltration is started; 4800 l of permeate are eliminated while maintaining the volume of the retentate constant by addition, at the same debit 4800 l of demineralised water at 45 ± 2° C.

The chloride content of the retentate is demineralised. It should not exceed 1.7 g/l. If it does, the ultrafiltration must be pursued by elimination of a supplementary volume of permeate till the chloride concentration has reached the desired level.

The retentate volume is adjusted to 12000 l employing demineralised water having a temperature of 45 ± 2° C.

The temperature and pH is verified; if necessary the temperature is readjusted by heating to 45 ± 2° C and the pH re-adjusted to pH 8 by addition of alkaline mixture B (Ex. 2.2 below).

1.5 Trypsin/Chymotrypsin Hydrolysis in Presence of Elastase

PEM (Ex. 2.3 hereinafter) and elastase (Ex. 2.4 hereinafter) are added simultaneously in the form of prepared solutions: PEM at a rate of 3.5 g enzyme per kg of protein substrate and elastase at 1120 U_2 and max. 3800 U_1 per kg of protein substrate.

The pH is maintained at pH 8 ± 0.1 during 1 h 15 employing alkaline neutralization solution B prepared in advance.

The hydrolysis is continued for a further 1 h 15 period without further pH adjustment. During the total PEM/elastase hydrolysis period, agitation of the mixture is continued and the temperature maintained at 45 ± 2° C.

1.6 Ultrafiltration/Diafiltration - Concentration

The protein hydrolysate solution is then subjected to ultrafiltration at 50° C, employing IRIS 3038 membranes (Rhône Poulence) - membrane surfaces 80 m^2.

The ultrafiltration is followed by a diafiltration when the refraction index of the retentate attains a value of ca. 13 %.

The diafiltration degree employed is 1.5.

The permeate is then cooled and concentrated till it has a refraction index of 35-40 %.

Residual proteins are removed by ultrafiltration on IRIS 3038 membranes (Rhône Poulenc), having a membrane surface of 80 m^2, at 50° C.

1.7 Sterilization / Drying

The concentrated permeate is sterilized (5 minutes at 130° C) and then spray dried (inlet temperature 180° C; outlet temperature 80° C).

The thus obtained product has the following physiochemical characteristics:

solubility	100 %
pH	6 %
dry extract	97.75 g/100 g
Proteins (N x 6.5)	89.34 g/100 g
Total nitrogen (TN)	13.74 g/100 g
Ashes	7.76 g/100 g
Ca	270 mg/100 g
Na	580 mg/100 g
K	2300 mg/100 g
Cl	2860 mg/100 g
N in free amino groups (AN)	2.66 g/100g
Total N content (NH_2 or NH) (TAN)	12.80 g/100 g.
Apparent degree of hydrolysis (AN/TN)	19.35 %
Effective degree of hydrolysis (AN/TAN)	20.75 %.

MW repartition (determined by UV detection at 215 nm)	
MW > 5000	3.3 %
5000 > MW > 3000	5.2 %
3000 > MW > 1000	33.9 %
1000 > MW > 500	30.9 %
500 > MW > 300	20.6 %
300 > MW	6.1 %

The aminogram (g/100 amino acids) of the thus obtained hydrolysate is as follows (average of 6 samples):

Lys	10.72 ± 0.47	Ala	5.24 ± 0.29
His	2.02 ± 0.14	Cys/Cystine	2.96 ± 0.76
Arg	2.58 ± 0.25	Val	4.23 ± 0.23
Asp	12.06 ± 0.66	Met	1.66 ± 0.09
Thr	4.67 ± 0.22	Ile	4.65 ± 0.66
Ser	3.74 ± 0.24	Leu	11.94 ± 0.67
Glu	18.47 ± 0.69	Tyr	3.04 ± 0.10
Pro	4.68 ± 0.55	Phe	3.63 ± 0.29
Gly	1.72 ± 0.33	Trp	2.24 ± 0.29

Free amino acid content (g/100 g product) - average of 3 samples:

Lys	0.72 ± 0.09	Gly	0.00 ± 0.00
His	0.01 ± 0.02	Ala	0.00 ± 0.00
Arg	0.15 ± 0.00	Cys/Cystine	0.00 ± 0.00
Asp	0.00 ± 0.00	Val	0.00 ± 0.00
Asn	0.00 ± 0.00	Met	0.01 ± 0.01
Thr	0.00 ± 0.00	Ile	0.05 ± 0.02
Ser	0.01 ± 0.01	Leu	0.26 ± 0.07
Glu	0.00 ± 0.00	Tyr	0.19 ± 0.11
Gln	0.00 ± 0.00	Phe	0.49 ± 0.17
Pro	0.00 ± 0.00	Trp	0.00 ± 0.00
		TOTAL	**1.89 ± 0.42**

Peptide composition (average of 6 samples):

Detection UV (215 nm)	Peptides with 2-5 amino acids:	28.80 ± 0.65
	Peptides with 2-10 amino acids:	60.83 ± 0.87
	Peptides with 4-10 amino acids:	39.52 ± 1.19
Detection Refr. Index	Peptides with 2-5 amino acids:	27.42 ± 3.78
	Peptides with 2-10 amino acids:	64.77 ± 1.92
	Peptides with 4-10 amino acids:	47.20 ± 2.67

EXAMPLE 2

2.1 Mixture A

To 60 l demineralised water at 25 + 2° C are added 30 kg crystalline citric acid, while stirring.

After complete dissolution of the citric acid, 30 l lactic acid (purity 80 %) are added. Then further demineralised water is added till a total volume of 130 l is obtained.

2.2 Mixture B

To 100 l demineralised water at 25 ± 2° C are added 52.97 kg KOH while stirring, till complete dissolution. Then 211.75 l pure 21 % ammonia are added and the volume adjusted to 350 l by addition of demineralised water.

2.3 PEM (3.5 g enzyme/kg of proteins)

The Proteolytic Enzyme Mixture employed is a concentrated mixture in powder form without salts, of porcine trypsin, bovine trypsin and bovine chymotrypsin, commercial designation P.E.M. 2500 S (NOVO Industrie Enzymes S.A. Paris), having a trypsine activity of at least 1800 USP-u/mg and a chymotrypsine activity of at least 350 USP-u/mg.

It is employed as an aqueous solution, by dissolution of PEM 2500 S in demineralised water at 25 ± 2° C.

2.4 Elastase (Pancreopeptidase E)

The elastase employed is from porcine pancreas origin, commercialised under Code E 3 by Biozyme (GB), in the form of a suspension in 70 % saturated ammonium sulphate with an activity of not less than 120 U/mg protein.

The unit definition employed by Biozyme is as follows: the amount of enzyme causing the hydrolysis of 1 micromole of N-acetyl-tri-L-alanine methyl ester per minute at 25° C and pH 8.5 (Method of assay: Gertler and Hofmann (1970) Can. J. Biochem. 48 384).

EXAMPLE 3 - Whey Protein Hydrolysate

One proceeds analogous to the procedure of Example 1, except that after the neutralization phase of 1h 15 (step 1.5) the hydrolysis is continued for another 1h 45 (instead of 1h 15 minutes).

The MW repartition is as follows: (determined by UV detection at 215 nm)

MW > 5000	3.4
5000 > MW > 3000	10.0
3000 > MW > 1000	34.9
1000 > MW > 500	28.9
500 > MW > 300	15.4
300 > MW	7.4

EXAMPLE 4 - Whey Protein Hydrolysate

One proceeds analogous to the procedure of Example 1, except that after the neutralization phase of 1h 15 (step 1.5), 1 % COROLASE 7092 is added and the hydrolysis continued for 1h 15.

COROLASE 7092 (Röhm) is a proteolytic enzyme preparation in liquid form from Aspergillus oryzae cultures having an activity of 2000 m UHb/mg, 1UHb being the enzyme quantity releasing with one minute at pH 7.5 and 37° C the equivalent of 1 micro mole tyrosine in TCE-soluble hydrolysate from haemoglobulin.

The thus obtained product has the following physiochemical characteristics:

Dry Extract	97.97 g/100 g
Total nitrogen content (kjeldahl)	13.30 g/100 g
Protein content (x 6.5)	85.01 g/100 g
Ashes	7.99 g/100 g
Calcium	0.33 g/100 g
Sodium	0.67 g/100 g
Potassium	3.05 g/100 g
Chlorides	2.72 g/100 g
pH	6.05
Solubility	100 %

Its MW repartition is as follows: (determined by UV detection at 215 nm)

MW > 5000	0.7
5000 > MW > 3000	2.0
3000 > MW > 1000	29.3
1000 > MW > 500	26.0
500 > MW > 300	29.3
300 > MW	12.7

EXAMPLE 5 - Whey Protein hydrolysate

One proceeds analogous to the procedure of Example 4, except that 1 % COROLASE PP, a pancreatic proteinase, is employed instead of COROLASE 7092.

EXAMPLE 6 - Whey Protein hydrolysate

One proceeds analogous to the procedure of Examples 1 to 4, except that the whey protein employed as a starting material is as follows pretreated to remove large proteins.

Commercially available delactosed lactoserum protein powder is purified and pretreated to remove macrolipids and most of large proteins having a molecular weight of more than 60'000 Daltons employing microfiltration and ultrafiltration techniques on membranes having a dynamic cut-off over 50'000.

The conditions are selected such that ca. 95 % of the immunoglobulins IgG are eliminated by microfiltration on membrane 0.22 micron, measured by immunotechnic and that the product comprises ca. 75 g proteins per litre and less than 2 g of lactose per litre.

EXAMPLE 7 - Trypsin/Chymotrypsin/Elastase-type 2 - hydrolysis of rennet casein

a) 1000 kg of rennet casein (obtained from milk by enzymic precipitation with rennet, comprising at least 84 % by weight of proteins-relative to the total dry material and having a water content of 10 % or less) are added portionwise with stirring to a reaction vessel of 12 m^3 comprising 7000 l of demineralised water, cooled at a temperature of 5° C. The mixture is rinsed with 700 l of demineralised water and the volume of the mixture adjusted to 8300 l. The hydration time is 1 hour.

b) The pH of the solution of step a) is adjusted to pH 8 ± 0.1 employing the alkaline solution [B] of ammonia and potassium hydroxide (according to Example 2.2).

c) The temperature is adjusted to 45° C ± 2° C.

d) PEM (see Ex. 2.3) and elastase (see Ex. 2.4) are added simultaneously to the thus obtained mixture: PEM at a rate of 1.96 g/kg of proteins, elastase at a rate of 3420 U_1 per kg of protein.

The pH is maintained at 8 ± 0.1 for 1 h 15 by neutralization of the solution employing the ammonia/potassium hydroxide solution B (Example 2.2).

e) COROLASE 7092 (Rhōm) is then added at a rate of 15 g/kg proteins. The mixture is kept at 45° ± 2° C for 1hr 15.

f) After the hydrolysis has been finalized (the total hydrolysis duration is 2h 30) the mixture is subjected to ultrafiltration employing IRIS 3038 membranes (Rhone-Poulenc) having a membrane surface of 80 m^2. The temperature is 50° C.

g) The ultrafiltration is followed by a diafiltration when the refraction index attains a value of about 13 %.

The diafiltration degree employed is 1.5.

The temperature of the permeate must be cooled to below 5° C if it is stored for more than 2 hours.

h) The permeate is concentrated by evaporation till the refraction index value is 35 to 40 %.

The concentrate is sterilized at 125° ± 2° C for 1 minute and then spray dried (inlet temp. 180° C; outlet temp. 80° C).

The thus obtained casein hydrolysate has the following characteristics:

Solubility	100 %
pH	7.26
Dry extract	97.66 g/100 g
Proteins (N x 6.5)	94.12 g/100 g
Total N content (TN)	14.45 g/100 g
Ashes	4.27 g/100 g
Calcium	370 mg/100 g
Na	50 mg/100 g
K	1240 mg/100 g
Cl	90 mg/100 g.
N in free amino groups (AN)	3.35 g/100 g
Total N content (NH or NH_2) (TAN)	12.40 g/100 g
Apparent degree of hydrolysis (AN/TN)	23.2 %
Effective degree of hydrolysis (AN/TAN)	27.0 %

Repartition of MW: (determination by UV detection at 215 nm)	
MW > 5000	0 %
5000 > MW > 3000	0.1 %
3000 > MW > 1000	18.6 %
1000 > MW > 500	37.4 %
500 > MW > 300	29.2 %
300 > MW	14.7 %

Free amino acid content 10.5 %.

Aminogram (in g/100 g Amino acids)							
Lys	7.9	Ala	2.7	His	3.0	Cys	0.5
Arg	3.8	Val	5.7				
Asp	6.7	Met	2.7				
Thr	3.3	Ile	3.8				
Ser	3.9	Leu	9.8				
Glu	20.7	Tyr	6.1				
Pro	10.7	Phe	5.6				
Gly	1.9	Trp	1.4				

Osmolarity mosmol/l	
50 g/l	119
100 g/l	236

Residual allergenicity: none

EXAMPLE 8 - Formulation for infants

Hydrolysed proteins *	13.1 g
Fat	20.2 g
Carbohydrates	58.4 g
Minerals	2.8 g
Non caloric organic substances**	0.7 g
Humidity	4.8 g
TOTAL	100 g

* (the 60:40 whey protein: casein mixture of which the whey protein is hydrolysed according to Example 1 after pretreatment according to Example 6 and the rennet casein is hydrolysed according to Example 7). The hydrolysate is supplemented

with 0.055 g L-cystine and 0.03 g of taurine per 100 g finished product.

** refers to the vitamin fraction and to the organic ligand component of certain trace elements and minerals of the formulation

Its aminogram is as follows (in g/100 g amino acid)

Asp/Asn	9.43	Met	2.24
Thr	4.59	Ile	5.33
Ser	4.17	Leu	11.80
Glu/Gln	18.65	Tyr	2.95
Pro	7.84	Phe	4.47
Gly	1.68	Lys	9.78
Ala	4.31	His	2.51
Cystine	0.71	Arg	2.90
Val	5.48	Trp	1.16

Claims

1. The use of whey protein hydrolysate substantially free of allergenic proteins for the manufacture of a dietary composition for the induction of cow's milk tolerance in children susceptible to cow's milk allergy.

2. The use according to Claim 1, wherein the whey protein hydrolysate is obtainable by hydrolysis of whey protein with trypsin/chymotrypsin in the presence of elastase 2.

3. The use according to Claim 1, wherein the whey protein hydrolysate is obtainable by prehydrolysis with pepsin, followed by hydrolysis of the whey protein prehydrolysate with trypsin/chymotrypsin in the presence of elastase 2.

4. The use according to Claim 1, wherein the whey protein hydrolysate is obtainable by prehydrolysis with pepsin, followed by hydrolysis of the whey protein prehydrolysate with trypsin/chymotrypsin in the presence of elastase 2 and - after an initial pancreatic hydrolysis phase - in the presence of the fungal proteolytic enzyme from Aspergillus oryzae having endo- and exopeptidase activity.

5. The use according to Claim 1, wherein the whey protein hydrolysate is obtainable by prehydrolysis with pepsin, followed by hydrolysis of the whey protein prehydrolysate with trypsin/chymotrypsin in the presence of elastase and - after an initial hydrolysis phase - in the presence of pancreatic proteinase extract.

6. The use according to Claims 2 to 5, wherein the whey protein employed as starting material is substantially free of macrolipids.

7. The use according to Claims 2 to 6, wherein the whey protein employed as starting material is substantially free of lactose.

8. The use according to Claims 2 to 7, wherein the whey protein employed as starting material is substantially free of proteins having a molecular weight of more than 60'000 Daltons.

9. The use according to Claims 1 to 8, wherein the dietary composition comprises as an additional protein source casein hydrolysate substantially free of allergenic proteins.

10. The use according to Claim 9, wherein the casein hydrolysate is obtainable by hydrolysis of casein with trypsin/chymotrypsin in the presence of elastase 2.

11. The use according to Claims 9 or 10, wherein the casein employed as starting material is substantially free of glycoprotein.

12. The use according to Claims 9 to 11, wherein the casein employed as starting material is rennet casein.

13. The use according to Claims 9 to 12, wherein the whey protein and casein hydrolysates are in a weight ratio whey protein hydrolysate: casein hydrolysate of 5:1 to 1:1.

14. The use according to Claims 1 to 13, wherein the dietary composition is in the form of a complete formula diet.

15. The use of whey protein hydrolysate substantially free of allergenic proteins for the manufacture of a dietary composition for the prophylaxis or treatment of type 1 diabetes mellitus in children susceptible to such disease.

16. The use according to Claim 15, wherein the whey protein hydrolysate is obtainable by hydrolysis of whey protein with trypsin/chymotrypsin in the presence of elastase 2.

17. The use according to Claim 15, wherein the whey protein hydrolysate is obtainable by prehydrolysis with pepsin, followed by hydrolysis of the whey protein prehydrolysate with trypsin/chymotrypsin in the presence of elastase 2.

18. The use according to Claim 15, wherein the whey protein hydrolysate is obtainable by prehydrolysis with pepsin, followed by hydrolysis of the whey protein prehydrolysate with trypsin/chymotrypsin in the presence of elastase 2 and - after an initial pancreatic hydrolysis phase - in the presence of the fungal proteolytic enzyme from Aspergillus oryzae having endo- and exopeptidase activity.

19. The use according to Claim 15, wherein the whey protein hydrolysate is obtainable by prehydrolysis with pepsin, followed by hydrolysis of the whey protein prehydrolysate with trypsin/chymotrypsin in the presence of elastase and - after an initial hydrolysis phase - in the presence of pancreatic proteinase extract.

20. The use according to Claims 16 to 19, wherein the whey protein employed as starting material is substantially free of macrolipids.

21. The use according to Claims 16 to 20, wherein the whey protein employed as starting material is substantially free of lactose.

22. The use according to Claims 16 to 21, wherein the whey protein employed as starting material is substantially free of proteins having a molecular weight of more than 60'000 Daltons.

23. The use according to Claims 15 to 22, wherein the dietary composition comprises as an additional protein source casein hydrolysate substantially free of allergenic proteins.

24. The use according to Claim 23, wherein the casein hydrolysate is obtainable by hydrolysis of casein with trypsin/chymotrypsin in the presence of elastase 2.

25. The use according to Claims 23 or 24, wherein the casein employed as starting material is substantially free of glycoprotein.

26. The use according to Claims 23 to 25, wherein the casein employed as starting material is rennet casein.

27. The use according to Claims 23 to 26, wherein the whey protein and casein hydrolysates are in a weight ratio whey protein hydrolysate: casein hydrolysate of 5:1 to 1:1.

28. The use according to Claims 15 to 27, wherein the dietary composition is in the form of a complete formula diet.

Figure 1

Fig. 1a

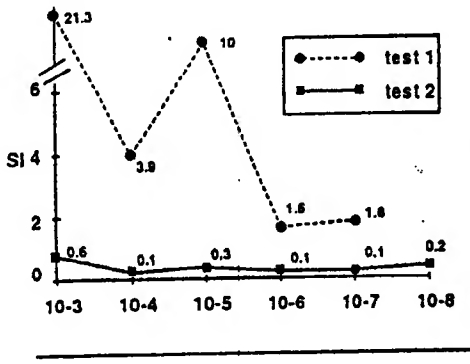


Fig. 1b

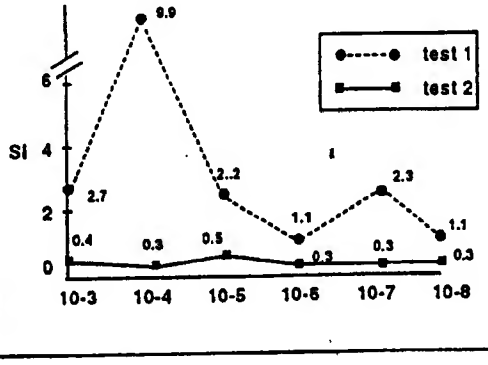


Fig. 1c

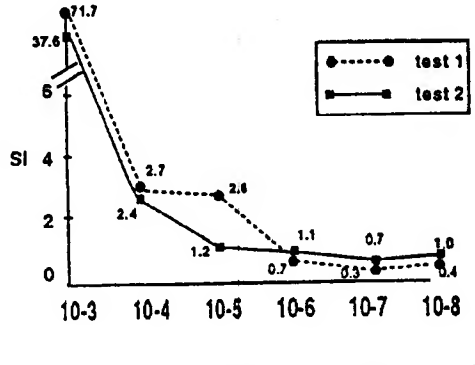


Fig. 1d

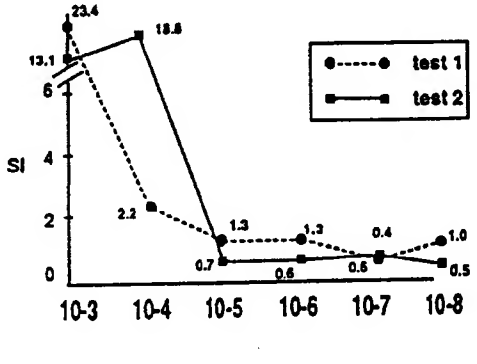


Figure 2

Fig. 2a

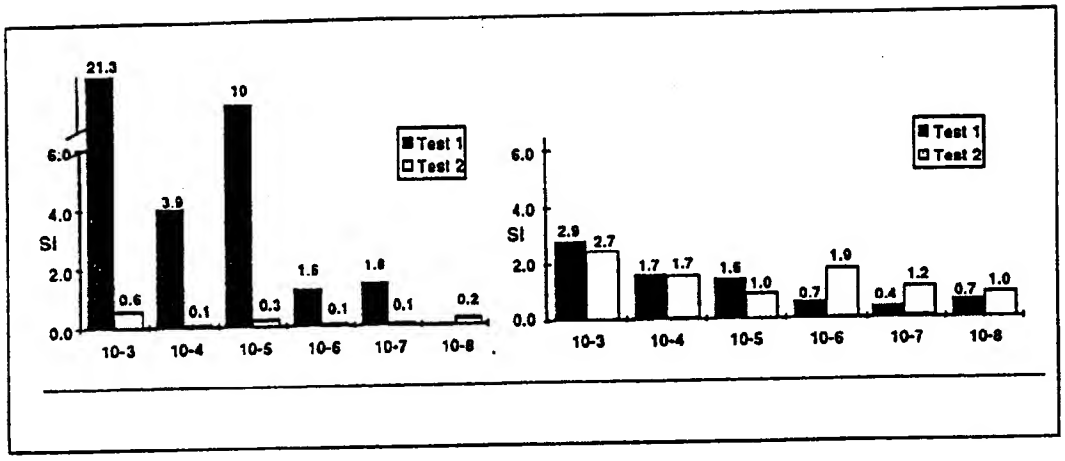


Fig. 2b

European Patent Office

EUROPEAN SEARCH REPORT

Application Number
EP 94 81 0352

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Y	* abstract * ---	16-28	
D,Y	EP-A-0 421 309 (SANDOZ NUTRITION LTD.) * claims 1,6,9,13,22,24,30; examples 6,7,10,14 * * page 5, line 21 - page 6, line 11; examples 18,20,24 * ---	2-14, 16-28	
A	PATENT ABSTRACTS OF JAPAN vol. 017, no. 266 (C-1062) 25 May 1993 & JP-A-05 005 000 (RIYOUSHIYOKU KENKIYUUKAI) 14 January 1993 * abstract * & DATABASE WPI Week 9307, Derwent Publications Ltd., London, GB; AN 93-055215 & JP-A-5 005 000 (RYOSHOKU KENKYUKAI) 14 January 1993 * abstract * --- -/--	1,2,8	

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A23J
A23L
A61K

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	23 September 1994	Kanbier, D

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
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A : technological background
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EPO FORM 1503 03.82 (P04C01)

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EUROPEAN SEARCH REPORT

Application Number
EP 94 81 0352

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.5)
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A	CHEMICAL ABSTRACTS, vol. 79, no. 19, 12 November 1973, Columbus, Ohio, US; abstract no. 114211, 'REMOVAL OF BITTER TASTE FROM PEPTIDE SOLUTION OBTAINED FROM MILK CASEIN AND WHEY' * abstract * & DATABASE WPI Week 7625, Derwent Publications Ltd., London, GB; AN 73-66177 & JP-A-48 052 967 (KIKKOMAN SHOYU CO. LTD.) 1973 * abstract * ---	4,5,18, 19	
A	WO-A-87 03785 (O.M. POULSEN) * page 6, line 26 - page 7, line 19; claims 1,10; example 2 * -----	5,19	TECHNICAL FIELDS SEARCHED (Int.Cl.5)

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	23 September 1994	Kanbier, D

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
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D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

EPO FORM 1503 03.82 (P04C01)

(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) EP 1 112 693 A1

(12) **EUROPEAN PATENT APPLICATION**

(43) Date of publication:
04.07.2001 Bulletin 2001/27

(51) Int Cl.[7]: **A23L 1/305**, A23L 1/29, A23L 1/09, A23L 2/38

(21) Application number: **99204607.8**

(22) Date of filing: **30.12.1999**

(84) Designated Contracting States:
AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE
Designated Extension States:
AL LT LV MK RO SI

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(54) **Composition comprising carbohydrate and peptide material and its use as an energy supplement after or during physical exercise or as a metabolic nutrient for oral consumption**

(57) The invention relates to a composition comprising carbohydrate and peptide material as well as an amount of at least one additional free amino acid selected from the group consisting of leucine and phenylalamine. This composition will enhance the blood insulin response after oral intake by humans and is intended for an enhanced recovery after physical exercise or to delay exhaustion during physical exercise.

EP 1 112 693 A1

Printed by Jouve, 75001 PARIS (FR)

Description

[0001] The invention relates to a composition comprising carbohydrate and peptide material, which enhance the blood insulin response after oral intake and intended for an enhanced recovery after physical exercise or to delay exhaustion during physical exercise. Further the invention relates to a metabolic nutrient for oral consumption.

Background of the invention

[0002] WO 97/39641 discloses an energy supplementation product in the form of a beverage or other nutrient for athletes or other persons in need of an increased glycogen level. This product is characterized by on the one hand a protein hydrolysate having a degree of hydrolysis (DH) of 1-50, preferably 15-30 and most preferably about 25 and on the other hand a carbohydrate like glucose, sucrose, maltose or a maltodextrine. In said WO 97/39641 it is stated that the intake of the energy supplementation product causes an increased insulin secretion enhancing the resynthesis of muscle glycogen. The rate of resynthesis of muscle glycogen after exercise is an important factor determining the time needed for recovery of the athlete. This is especially important for athletes involved in intensive exercise on a daily basis. However, it appeared that after exercise of the athlete to exhaustion a protein hydrolysate will not enhance significantly the plasma insulin response upon a carbohydrate load.

Objects and Summary of the Invention

[0003] It is an aim of the present invention to provide a good tasting and refreshing composition that can be taken orally and that stimulates the plasma insulin response. Taken after exercise the resulting enhanced insulin response highly stimulates muscle glycogen synthesis and thus recovery. Furthermore, protein anabolism in skeletal muscles is stimulated. Taken during exercise an enhanced uptake of glucose by the muscles would occur. This aim may be realised by providing a composition comprising carbohydrate and peptide material and an amount of at least one additional free amino acid selected from the group consisting of leucine and phenylalamine.

Detailed description of the invention

[0004] As indicated above the composition according to the invention comprises carbohydrate and peptide material and at least one of the additional free amino acids leucine and/or phenylalamine, preferably both. Said additional free amino acids are each present in an amount of 0.2-20 wt.%, preferably 1-10 wt.%, calculated on the dry weight of the composition.

[0005] Next to the above additional free amino acids it is possible to use the further additional free amino acids arginine and/or glutamine which each may be present in an amount in the range of 0.1-20 wt.% calculated on the dry weight of the composition.

[0006] The peptide material can be derived from proteins of animal or plant origin and examples of such proteins are milk proteins, meat proteins, soy proteins, wheat proteins, pea proteins, rice proteins and maize proteins. Preferably the protein raw material is wheat gluten protein or a subfraction thereof such as gliadin. In the present context, the term "peptide material" is understood to indicate a protein hydrolysate and may contain all types of peptides that may vary in length as well as a certain amount of free amino acids resulting from the hydrolysis. The protein raw material is hydrolysed by one or more hydrolytic enzymes. The hydrolytic enzyme can be of animal, plant, yeast, bacterial or fungal origin. Preferably enzyme preparations are used which have a low exo-peptidase activity to minimise the liberation of free amino acids and to improve taste profiles of the protein hydrolysates. The preferred hydrolysed protein material of the present invention has an average peptide chain length in the range of 2-40 amino acid residues and more preferably in the range of 3-20 amino acid residues. The average peptide chain can be determined using the method as described in WO 96/26266. The protein hydrolysates that can be used to prepare a composition as disclosed in the present invention are not limited to ones disclosed in the present invention but include all protein hydrolysates that can be obtained by enzymatic hydrolysis using common techniques as described in the literature and known to those skilled in the art. Further the peptide material is present in an amount of 0.1-50 wt.%, preferably 2-25 wt.%, calculated on dry matter basis of the composition.

[0007] The carbohydrate material component of the composition according to the invention is advantageously selected from the group consisting of mono-, di- and polysaccharides like glucose, sucrose, maltose as well as more complex edible carbohydrates such as maltodextrines. Independent on the type of carbohydrate material it is present in an amount in the range of 10-90 wt.%, preferably 50-80 wt.%, calculated on dry matter basis of the composition.

[0008] Other optional components of the composition according to the invention are vitamins, minerals, flavours, antioxidants, components having co-enzyme and antioxidant properties, lipids including emulsifiers, and proteins for meeting specific nutritional and/or physiological needs.

[0009] The composition according to the invention may have the form of a powder, a beverage or any other food product. A beverage according to the invention can be prepared by dissolving the above-defined ingredients in an appropriate amount of water. Preferably an isotonic drink has been prepared. For drinks, intended to be used during and after exercise it is recommended to have a concentration of the composition according to the invention in the range of 10-15 wt.% calculated on the total weight of the drink.

[0010] In view of the complexicity of the processes dealing with the recovery of athletes after (exhaustive) exercise the following is remarked.

[0011] Athletes undergoing intense, prolonged training or participating in endurance races (e.g. the marathon) easily catch a cold or flu. This is most probably related to the significant decreased plasma levels of the amino acid glutamine seen during recovery after exercise at exhaustion. A marked increase in numbers of white blood cells occurred immediately after exhaustive exercise, followed by a decrease in the numbers of lymphocytes. The amino acid glutamine is essential for the optimal functioning of a number of tissues in the body, particularly of the immune system and the gut. The provision of oral glutamine after exercise appeared to have a beneficial effect on the level of subsequent infections. In addition, the activity of T-lymphocytes appeared to be increased in samples from those who received glutamine compared with placebo. If recovery between exercise bouts is inadequate, the acute effects of exercise on plasma glutamine level may be cumulative, since overload training has been shown to result in low plasma glutamine levels requiring prolonged recovery. Plasma glutamine level may be useful as an indicator of an overtrained state. As free glutamine is not stable in solution during pasteurisation and during storage, glutamine-containing peptides are the preferred glutamine source. Glutamine containing peptides can be obtained from the hydrolysis of vegetable and animal proteins, a preferred protein source is wheat gluten since this is rich in glutamine. Infections also are an important cause of morbidity and mortality in patients with multiple trauma. Studies in both animals and human beings have suggested that glutamine-enriched nutrition decreases the number of infections. In patients with multiple trauma receiving glutamine-supplemented enteral nutrition a low frequency of pneumonia, sepsis, and bacteraemia was seen.

[0012] In addition to its direct action on the cells of the immune system, glutamine may indirectly influence the immune system by the preservation of action of the antioxidant glutathione. The tripeptide glutathione (GSH) is the major intracellular antioxidant and is essential to normal cell function and replication. Studies over the last decade have demonstrated that glutamine becomes essential during metabolic stress to replete tissue GSH levels which have become depleted. The availability of glutamine appears to be important for the regeneration of GSH stores. Due to the high intake of oxygen during physical exercise there is an increased production of radicals and other forms of reactive oxygen species (ROS) in the muscle. ROS has been implicated as an underlying cause in exercise-induced disturbances in muscle homeostasis (e.g. redox status), that could result in muscle fatigue or injury. Important nonenzymatic antioxidant defences include GSH, vitamin E, vitamin C, α-lipoic acid, carotenoids, polyphenols including flavonoids and isoflavones, uric acid, bilirubin, and ubiquinone. α-Lipoic acid functions as a cofactor for α-dehydrogenase complexes and participates in the oxidative decarboxylation of α-keto acids such as pyruvate, α-ketoglutarate and branched chain α-keto acids. Normally, α-lipoic acid is present in small quantities in animal tissues and is generally bound to an enzyme complex and is therefore unavailable as an antioxidant. However, exogenous free unbound α-lipoic acid may be effective as an antioxidant and an recycling vitamin C, which increases the intracellular GSH concentration. Dietary supplementation with antioxidants has been shown to be beneficial in combating oxidative stress without enhancing performance while GSH levels were found to influence the endurance capacity of athletes.

[0013] A further aspect of the glucose uptake during and after exercise is elucidated below. In fact numerous factors determine the rate of glucose uptake during and after exercise. During exercise, one of the most important regulatory responses is an increase in blood flow to the contracting skeletal muscles. This increased blood flow provides ample substrate to the working muscles, and thus, glucose availability is usually not the rate-limiting factor for glucose utilisation. Instead, glucose transport is thought to be the rate-limiting step in glucose during exercise. Glucose transport occurs primarily by facilitated diffusion, an energy-independent process that uses GLUT-4, the major glucose carrier in human and rat skeletal muscle for transport of glucose across the plasma membrane. Both exercise and insulin increase glucose transport through an increase in the maximal velocity of transport. This increase in transport may occur through an increase in the rate that each GLUT-4 protein transport glucose (transport turnover number), an increase in the number of functional glucose transporter proteins present in the plasma membrane, or both. It appears that exercise and insulin recruit distinct GLUT-4-containing vesicles and/or mobilise different "pools" of GLUT-4 proteins in skeletal muscle originating from unique intracellular locations. The combined intake of carbohydrates and protein hydrolysates, peptides and/or amino acids will enhance the uptake of glucose during exercise by recruiting different GLUT-4 proteins to the plasma membrane of the contracting muscle cells. As a result the performance of the active muscles is enhanced and exhaustion will be delayed.

Experimental

[0014] In studies presented below the effects of intact casein, several protein hydrolysates and protein hydrolysate

in combination with specific amino acids have been examined. The sodium casein used in this study is commercially available from DMV-International. Glucose and maltodextrin were obtained from AVEBE (the Netherlands) and crystalline amino acids from BUFA (the Netherlands). In the presented study the following commercially available protein hydrolysates from Quest-International have been used: Hyprol® 3301 (whey protein hydrolysate, average peptide chain length of 4.1), Hyprol® 4107 (wheat gluten protein hydrolysate, average peptide chain length of 12.2) and Hyprol® 7102 (pea protein hydrolysate, average peptide chain length of 6.4). The insulin responses in blood plasma was analysed by radio-immuno-assay (Insulin RIA 100 kit, Pharmacia, Sweden).

[0015] in three studies the efficiency of an amino acid and/or protein (hydrolysate) mixture in a carbohydrate containing drink with respect to their insulinotropic effect in human subjects was examined. The composition of all the tested experimental drinks are given in table 1.

First study

[0016] The aim of the first study was to establish an amino acid and/or protein (hydrolysate) mixture with a maximal insulinotropic effect when co-ingested with carbohydrates.

[0017] Eight healthy, non-obese male subjects (age 21±0.4, weight 73.9±2.2 kg, height 186±2 cm, BMI 21.4±0.7 $kg \cdot m^{-2}$) after an overnight fast, were tested for 2 hours on 10 occasions in which different beverage compositions were ingested. During those trials subjects ingested 0.8 $g \cdot kg\ bw^{-1} \cdot h^{-1}$ carbohydrate and 0.4 $g \cdot kg\ bw^{-1} \cdot h^{-1}$ of an amino acid and/or protein (hydrolysate)-mixture. When the mixture of free amino acids and protein hydrolysate was tested, 0.2 $g \cdot kg\ bw^{-1} \cdot h^{-1}$ wheat gluten protein hydrolysate, 0.1 $g \cdot kg\ bw^{-1} \cdot h^{-1}$ leucine and 0.1 $g \cdot kg\ bw^{-1} \cdot h^{-1}$ phenylalanine was consumed. The drinks were ingested at a rate of 3.5 $ml \cdot kg\ bw^{-1}$ per half-hour. A strong initial increase in plasma glucose and insulin levels was observed in all trials after which large differences in insulin response between drinks became apparent. The insulin response is expressed as area under the curve during the second hour. It was found that the ingestion of the drinks containing free leucine, phenylalanine and arginine and the drinks with free leucine, phenylalanine and wheat protein hydrolysate was followed by the largest insulin response (201 and 203%, respectively; $P<0.05$) compared to the carbohydrate-only drink (see Table 1). The insulin responses correlated positively with plasma leucine, phenylalanine and tyrosine levels. The positive correlation observed with plasma tyrosine levels may be explained by the fact that the amino acid tyrosine is the hydroxylation product of phenylalanine in the liver and is formed when large amounts of phenylalanine are ingested. Ingestion of a test drink containing large amounts of free arginine (0.4 g arginine·kg $bw^{-1} \cdot h^{-1}$) caused severe diarrhoea and the urge to defecate in all subjects for several hours during and after the trial. These gastrointestinal problems appeared to prevent intestinal absorption of the arginine as lower concentrations of arginine were seen in plasma following ingestion of other arginine containing test drinks. This indicates that in sports practice it would not be recommendable to ingest large amounts of arginine in order to stimulate growth hormone release and muscle anabolism.

[0018] The addition of glutamine to the mixture of arginine, leucine and phenylalanine had no effect on the insulin response; this suggests that, at least in the studied healthy men, *in vivo* enough glutamine is present, at least in the studied healthy men (600-800 $\mu mol \cdot l^{-1}$ in plasma). Also the addition of free glutamine hardly influenced plasma glutamine concentrations. The drink containing the wheat gluten protein hydrolysate (drink 5) gave the highest insulin response of all tested protein hydrolysates. Although no statistical significant differences were found between the insulin responses in test drinks containing whey, pea and wheat hydrolysate vs. the control carbohydrate-only trial, the mean insulin responses were 155, 125 and 181%, respectively, compared to the control trial. There were no differences in plasma leucine and phenylalanine responses between the different protein hydrolysates tested. None of the hydrolysates gave rise to gastrointestinal or other complaints. Furthermore, the insulin responses on the ingestion of the drink containing the free amino acids leucine, phenylalanine and arginine (drink 6), the drink containing the mentioned three amino acids as well as glutamine (drink 7), as well as the drink containing wheat gluten protein hydrolysate and the free amino acids leucine and phenylalanine (drink8) were the same (table 1).

[0019] The main conclusion is that oral intake of amino acids in combination with carbohydrates can result in an insulinotropic effect as large as 200% compared to the intake of carbohydrates only. Furthermore, a mixture of free leucine, phenylalanine and arginine can produce a large insulinotropic effect when ingested in combination with carbohydrates. Surprisingly, the addition of leucine and phenylalanine to a wheat gluten protein hydrolysate created a similar insulinotropic effect as the drinks containing arginine (drink 6) but without any gastrointestinal discomfort. Following the ingestion of the intact protein (drink 2) plasma amino acid responses were in general lower compared to the responses observed following ingestion of protein hydrolysates. Therefor the use of protein hydrolysates is preferred in order to stimulate insulin secretion. Another practical disadvantage of the use of an intact protein when ingested as a drink is its poor solubility in water.

Second study

[0020] In the second study the correlation between glucose and insulin responses after oral intake of the composition of the first study (wheat gluten protein hydrolysate, free leucine, phenylalanine and carbohydrate) with respect to the post-exercise muscle glycogen synthesis was examined. This study investigated whether an increase in carbohydrate intake and/or ingestion of a protein hydrolysate/amino acid mixture in combination with carbohydrate can increase post-exercise muscle glycogen synthesis rates when compared to the ingestion of 0.8 g·kg $bw^{-1}·h^{-1}$ carbohydrate, provided at 30-min intervals. (In Appl. Physiol., 1988 64(4) 1480 it is reported that in healthy athletes a maximum glycogen resynthesis rate is obtained upon ingestion of about 0.75 g·kg $bw^{-1}·h^{-1}$).

[0021] Eight trained cyclists (age: 24.0±0.6 years, body mass: 70.0±1.0 kg, BMI: 21.4±0.6 $m·kg^{-2}$) visited the laboratory 3 times during which a control and 2 other beverage compositions were tested. The subjects were subjected to a glycogen depletion protocol in which they cycled in two-minute block periods at alternating workload of 90 and 50% of their maximum performance capacity (maximum workload (Wmax): 390±8 W, maximum heart rate: 191±3 bts min^{-1}). This was continued until the subjects were no longer able to complete the two-minutes at 90% of their maximum. Subjects were allowed to stop when pedalling speed could not be maintained at 70% of their maximum capacity. After they had stopped muscle biopsy samples were collected and subjects received a beverage every 30 min to ensure ingestion of 0.8 g·kg $bw^{-1}·h^{-1}$ carbohydrate (CHO, drink 1), 0.8 g·kg $bw^{-1}·h^{-1}$ carbohydrate + 0.4 g·kg $bw^{-1}·h^{-1}$ wheat protein hydrolysate + free leucine and phenylalanine (CHO+PRO, drink 8) or 1.2 g·kg $bw^{-1}·h^{-1}$ carbohydrate (CHO+CHO, drink 10). After 5 hours a second biopsy was taken. Plasma insulin responses in the CHO+PRO (drink 8) and CHO+CHO trial (drink 10) were increased (table 1) compared to the CHO trial (drink 1) (+88±17 and +46±18 % respectively; $P<0.05$). Muscle glycogen synthesis was increased in both treatments compared to the CHO trial (+35.4±5.1 and +44.8±6.8 vs. 16.6±7.8 µmol glycosyl units·g $dw^{-1}·h^{-1}$, respectively: $P<0.05$).

[0022] Surprisingly, the high carbohydrate drink (CHO+CHO, drink 10) stimulated the highest glycogen synthesis in skeletal muscle but the CHO+PRO (drink 8) has the highest plasma insulin response. This suggests that the amount of glucose is limiting in the drink 8, which is the same as the control (drink 1), the amount of glucose is limiting for glycogen synthesis and indicates that post exercise ingestion of 0.8 g·kg $bw^{-1}·h^{-1}$ carbohydrate is not the maximum as is generally accepted with respect to glucose absorption as is generally accepted. More glucose can be absorbed as is shown by drink 10 which provides an intake of even 1.2 g·kg $bw^{-1}·h^{-1}$ carbohydrate.

Third study

[0023] To investigate the insulinotropic effect of protein hydrolysates and leucine and phenylalanine in combination at the high carbohydrate intake of 1.2 g·kg $bw^{-1}·h^{-1}$, a third study was performed in highly trained athletes. Here, the post-exercise insulin response as well as the plasma amino acid response following the combined ingestion of carbohydrate and wheat gluten protein hydrolysate with and without the addition of free leucine and phenylalanine in trained athletes was examined. After an overnight fast, 8 male cyclists (age: 24.0±0.6 years, body mass: 70.0±1.0 kg, BMI: 21.4±0.6 $m·kg^{-2}$) on 5 occasions were subjected to a glycogen depletion protocol. Thereafter a control drink and 2 different beverage compositions in 2 different doses were tested. After performing the glycogen depletion protocol (see 2nd study) subjects received a beverage volume of 3.5 ml·kg bw^{-1} every 30 minutes to ensure an intake of 1.2 g·kg $bw^{-1}·h^{-1}$ carbohydrate and 0, 0.2 or 0.4 g·kg $bw^{-1}·h^{-1}$ protein hydrolysate/amino acid mixture. The insulin response is expressed as area under the curve. It was found that the ingestion of the beverages containing wheat hydrolysate, free leucine and phenylalanine resulted in a substantial increase in insulin response (+52 and +107%, respectively; $P<0.05$) compared to the control (carbohydrate only) trial (table 1). A dose related effect exists as doubling the dose (0.2 to 0.4 g·kg $bw^{-1}·h^{-1}$) lead to an additional rise in insulin response ($P<0.05$).

[0024] In contrast to our first study with subjects after an overnight fast, we found no significant increase in post-exercise insulin response following the ingestion of a wheat gluten protein hydrolysate at an intake of 0.2 or 0.4 g·kg $bw^{-1}·h^{-1}$ in combination with carbohydrate at 1.2 g·kg $bw^{-1}·h^{-1}$ compared with the control (carbohydrate-only) drink. This can partly be explained by the higher carbohydrate intake (1.2 vs. 0.8 g·kg $bw^{-1}·h^{-1}$) that was applied in the third study. Furthermore, this third study was performed following intense exercise and the insulin response is likely to be reduced as muscle contraction stimulates glucose transport, largely mediated by translocation of GLUT4 from intracellular sites to the plasma membrane as discussed earlier. Surprisingly we found that a substantial increase in insulin response is seen following the ingestion of the mixtures containing wheat gluten protein hydrolysate together with free leucine and phenylalanine when compared to the control ($P<0.05$). Ingestion of 0.2 and 0.4 g·kg $bw^{-1}·h^{-1}$ of this mixture in combination with carbohydrate resulted in an additional increase in insulin response of 51.8±9.5 and 107.4±16.7%, respectively compared to the control trial ($P<0.05$).

[0025] As both glucose availability and insulin concentrations determine the rate of glucose uptake in skeletal muscle, increasing postexercise insulin levels could have practical importance for the optimisation of glycogen synthesis rates and protein metabolism in skeletal muscle.

Table 1.

The values in the table are given in gram dry product per 100 ml drink.															
Composition of used test drinks and their insulinotropic effects.															
Test drink	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8***	**9***	**10**	**11**	**12**	**13**	**14***	**15***
Intact casein		5.71													
Whey protein hydrolysate			5.71												
Pea protein hydrolysate				5.71											
Wheat protein hydrolysate					5.71			2.86	2.86			2.86	5.71	1.43	2.86
Leucine						1.90	1.43	1.43	0.95					0.71	1.43
Phenylalanine						1.90	1.43	1.43	0.95					0.71	1.43
Arginine						1.90	1.43		0.95						
Glutamine							1.43								
Glucose	5.71	5.71	5.71	5.71	5.71	5.71	5.71	5.71	5.71	8.57	6.85	6.85	6.85	6.85	6.85
Malto-dextrin	5.71	5.71	5.71	5.71	5.71	5.71	5.71	5.71	5.71	8.57	10.28	10.28	10.28	10.28	10.28
Sodium saccharinate	0 02	0.02	0.02	0.02	0 02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Citric acid	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18
Cream vanilla flavour	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Test drink	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8***	**9***	**10**	**11**	**12**	**13**	**14***	**15***

* drinks according to the invention

Table 1. (continued)

Composition of used test drinks and their insulinotropic effects.															
Test drink	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8***	**9***	**10**	**11**	**12**	**13**	**14***	**15***
1st Study Healthy male subjects Overnight fast	Plasma insulin response (area under curve minus baseline values): mean ± SEM (mU·ml^{-1}·2hrs)**														
	4.61 ± 0.68	5.10 ± 1.44	6.64 ± 1.01	5.15 ± 0.37	7.33 ± 1.19	7.24 ± 1.15	7.16 ± 1.49	7.10 ± 0.59	6.56 ± 0.86						
	Plasma insulin response (area under curve minus baseline values): mean ± SEM (mU·ml^{-1}·2nd hr)***														
	2.53 ± 0.37	3.30 ± 1.05	3.93 ± 0.56	3.16 ± 0.17	4.59 ± 0.86	5.08 ± 0.88	4.61 ± 0.96	5.14 ± 0.35	4.28 ± 0.62						
2nd Study Male athletes Post exercise	Plasma insulin response (area under curve minus baseline values): mean ± SEM (mU·ml^{-1}·5hrs)														
	8.58 ± 0.86							15.89 ± 2.21		12.27 ± 1.84					
3rd Study Male athletes Post exercise	Plasma insulin response (area under curve minus baseline values): mean ± SEM (mU·ml^{-1}·3hrs)														
											5.91 ± 1.02	5.82 ± 0.42	5.83 ± 0.60	8.42 ± 0.78	11.32 ± 1.00

* drinks according to the invention

** average over two hours

*** average over the second hour

[0026] In case of the dry powder version of the composition of the present invention it is preferred to use agglomerated ingredients or to agglomerate the whole composition in order to facilitate the rehydration process. The following, non-limiting examples illustrate the embodiments according to the invention.

Example 1.

[0027] The composition as shown in table 2 was chosen such that the finally obtained drink is isotonic. Hyprol® 4107 is a wheat gluten protein hydrolysate from Quest-International. One litre of drink was prepared by dissolving 177.4 gram of the powder in an appropriate amount of water. The drink was found to be good tasting and refreshing.

Table 2.

Composition of tropical sports drink.		
Ingredients	**Powder composition g/kg**	**Composition of drink %**
Maltodextrin	430.70	7.64
Sugar extra fine	225.12	3.99
Hyprol® 4107	140.93	2.50
Leucine	70.47	1.25
Phenylalanine	70.47	1.25
Aspartame	1.40	0.02
Citic acid powder	37.21	0.66
Orange Flavour	5.21	0.09
Tropical Flavour	6.79	0.12
Carotenoids	0.56	0.01
Clouding agent	7.44	0.13
Tricalcium phosphate	3.72	0.07
Total	1000.00	17.74

Example 2.

[0028] A composition as shown in table 3 was chosen such that the finally obtained drink is isotonic. Hyprol® 4107 is a wheat gluten protein hydrolysate from Quest-International. One litre of drink was prepared by dissolving 165 gram of the powder in an appropriate amount of water. The drink was found to be good tasting and refreshing.

Table 3.

Composition of strawberry sports drink		
Ingredients	**Powder composition g/kg**	**Composition of drink %**
Maltodextrin	445.18	7.39
Sugar extra fine	221.69	3.68
Hyprol® 4107	150.60	2.50
Leucine	75.30	1.25
Phenylalanine	75.30	1.25
Aspartame	1.82	0.03
Citric acid	19.88	0.33
Strawberry flavour	1.82	0.03
Caramel	1.21	0.02
Cochineal (colour)	1.82	0.03
α-Lipoic acid	3.61	0.06
Vitamin C	1.21	0.02
Isoflavones (soy)	0.60	0.01
Total	1000.0	16.60

Example 3

[0029] Stable liquid enteral feeding for use in hospital and at home, containing both protein hydrolysate and fat (5%). Hyprol® 7102 is a pea protein hydrolysate from Quest-International. Also vitamins and calcium are added. The anti-oxidants α-lipoic acid, flavonoids, carotenoids and the vitamins E and C are included.

Table 4.

Composition of liquid enteral feeding	
Ingredients	**Composition of drink**
30% fat emulsion NN-26751	175.00 g
Maltodextrine 27057	90.00 g
Aspartame	0.20 g
Citric acid solution 50% w/w	3.20 ml
Ascorbic acid	0.20 g
Vegetable protein Hyprol® 7102. 5 Z10418	20.00 g
Leucine	10.00 g
Phenylalanine	10.00 g
Calcium lactate 5 aq.	3.90 g
Vitamin pre-mix 961*	0.20 g
Orange Compound Coloured QL-2319	22.50 g
Tropical NN-20325	1.50 g
α-Lipoic acid	0.60 g
Flavonoids (grape seed extract)	0.20 g
Colours (E160 a + E 160 e)	8 mg
With water up to	1000 ml
* At the given dosage, the vitamin mix contains the RDA amounts for Vitamins B_1, B_2, B_3, B_5, B6, B_{11}, B_{12}, C, E, and H per litre final drink	

Example 4

[0030] Nougat bar centre for a sports bar. The casein hydrolysate Hyfoama® DSN and the wheat gluten protein hydrolysate Hyprol® 4107 are both products from Quest-International. The nougat bar centres makes about 40% in weight from a sports bar, 20% can be caramel and 40% of the bar exists out of a chocolate outside.

Table 5.

Composition of nougat sports bar centre	
Ingredient	**Composition**
Sugar	375 g
Glucose (35DE)	359 g
Icing sugar	11 g
Skimmed milkpowder	43 g
Hyprol® 4107	20 g
Leucine	10 g
Phenylalanine	10 g
Hyfoama ®DSN	3.2 g
Egg albumen (powder)	2.1 g
Molten fat (hydrogenated coconut or palmkernel)	43 g
Flavour (dissolved in fat)	2.6 g
α-Lipoic acid	1.5 g
Vitamin C	0.5 g

Table 5. (continued)

Composition of nougat sports bar centre	
Ingredient	**Composition**
Cocoa powder	43 g
Water	76 g
Total	1000 g

Claims

1. Composition comprising carbohydrate and peptide material and an amount of at least one additional free amino acid selected from the group consisting of leucine and phenylalamine.

2. The composition according to claim 1, wherein both the additional free amino acids leucine and phenylalamine are present.

3. The composition according to claim 1 or 2, wherein the additional free amino acids leucine and phenylalamine are each present in an amount in the range of 0.2-20 wt.%, preferably 1-10 wt.% calculated on dry matter basis.

4. The composition according to any of claims 1-3, wherein a further additional free amino acid, selected from the group consisting of arginine and glutamine is present.

5. The composition according to claim 4, wherein each of the further additional free amino acids arginine and glutamine is present in an amount in the range of 0.1-20 wt.%, calculated on dry weight basis.

6. The composition according to any of the claims 1-5, wherein the peptide material is derived from wheat proteins, rice proteins, pea proteins, casein proteins, whey proteins or mixtures thereof.

7. The composition according to any of the claims 1-6, wherein the peptide material is obtained by hydrolysis of protein material.

8. The composition according to claim 7, wherein the peptide material is derived from wheat protein.

9. The composition according to any of the claims 6-8, wherein the peptide material has an average peptide chain length in the range of 2-40 amino acids, preferably 3-20 amino acids.

10. The composition according to any of the claims 1-9, wherein the peptide material is present in an amount in the range of 0.1-50 wt.%, preferably 2-25 wt.%, calculated on dry matter basis.

11. The composition according to any of the claims 1-10, wherein the carbohydrate material is selected from the group consisting of mono-, di-, oligosaccharide and more complex edible carbohydrates such as maltodextrines.

12. The composition according to any of the claims 1-11, wherein the carbohydrate material is present in an amount of 10-90 wt.%, preferably 50-80 wt.%, calculated on dry matter basis.

13. The composition according to any of the claims 1-12, wherein vitamines, flavours, minerals, components having co-enzyme and antioxidant properties, lipids including emulsifiers, and proteins are present.

14. The composition according to any of the claims 1-12, wherein the composition has the form of an isotonic beverage or sports bar.

15. Use of the composition according to any of the claims 1-14 during or after physical exercise.

16. Use of the composition according to any of the claims 1-14 as an enteral clinical feeding.

European Patent Office

EUROPEAN SEARCH REPORT

Application Number
EP 99 20 4607

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The present search report has been drawn up for all claims

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X : particularly relevant if taken alone
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EUROPEAN SEARCH REPORT

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Place of search	Date of completion of the search	Examiner
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(19) Europäisches Patentamt
European Patent Office
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(11) EP 1 112 693 B1

(12) **EUROPEAN PATENT SPECIFICATION**

(45) Date of publication and mention of the grant of the patent:
22.03.2006 Bulletin 2006/12

(51) Int Cl.:
A23L 1/305 (2006.01) *A23L 1/29* (2006.01)
A23L 1/09 (2006.01) *A23L 2/38* (2006.01)

(21) Application number: **99204607.8**

(22) Date of filing: **30.12.1999**

(54) **Composition comprising carbohydrate and peptide material and its use as an energy supplement after or during physical exercise or as a metabolic nutrient for oral consumption**

Aufbaupräparat, das Kohlenhydrat- und Peptidmaterial enthält und sein Gebrauch als Energieergänzung nach oder während körperlicher Übung oder als metabolischer Nährstoff für die orale Verabreichung

Composition comportant le matériel d'hydrate de carbone et de peptide et son utilisation comme supplément d'énergie après ou pendant l'exercice physique ou comme aliment métabolique pour l'administration orale

(84) Designated Contracting States:
AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE

(43) Date of publication of application:
04.07.2001 Bulletin 2001/27

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- **DATABASE WPI Section Ch, Week 198645 Derwent Publications Ltd., London, GB; Class D13, AN 1986-294476 XP002137952 & JP 61 215323 A (OTSUKA PHARM CO LTD), 25 September 1986 (1986-09-25)**

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EP 1 112 693 B1

Description

[0001] The invention relates to a composition comprising carbohydrate and peptide material, which enhance the blood insulin response after oral intake and intended for an enhanced recovery after physical exercise or to delay exhaustion during physical exercise. Further the invention relates to a metabolic nutrient for oral consumption.

Background of the invention

[0002] WO 97/39641 discloses an energy supplementation product in the form of a beverage or other nutrient for athletes or other persons in need of an increased glycogen level. This product is characterized by on the one hand a protein hydrolysate having a degree of hydrolysis (DH) of 1-50, preferably 15-30 and most preferably about 25 and on the other hand a carbohydrate like glucose, sucrose, maltose or a maltodextrine. In said WO 97/39641 it is stated that the intake of the energy supplementation product causes an increased insulin secretion enhancing the resynthesis of muscle glycogen. The rate of resynthesis of muscle glycogen after exercise is an important factor determining the time needed for recovery of the athlete. This is especially important for athletes involved in intensive exercise on a daily basis. However, it appeared that after exercise of the athlete to exhaustion a protein hydrolysate will not enhance significantly the plasma insulin response upon a carbohydrate load.

Objects and Summary of the Invention

[0003] It is an aim of the present invention to provide a good tasting and refreshing composition that can be taken orally and that stimulates the plasma insulin response. Taken after exercise the resulting enhanced insulin response highly stimulates muscle glycogen synthesis and thus recovery. Furthermore, protein anabolism in skeletal muscles is stimulated. Taken during exercise an enhanced uptake of glucose by the muscles would occur. This aim may be realised by providing a composition comprising carbo-hydrate and peptide material and an amount of at least two additional free amino acids selected from the group consisting of leucine and phenylalanine, wherein the peptide material is obtainable by hydrolysis of protein material, the peptide material is derived from wheat protein, and the additional free amino acids leucine and phenylalanine are each present in an amount in the range of 0.2-20 wt.%, calculated on dry matter basis.

Detailed description of the invention

[0004] As indicated above the composition according to the invention comprises carbohydrate and peptide material and at least the additional free amino acids leucine and phenylalanine. Said additional free amino acids are each present in an amount of 0.2-20 wt.%, preferably 1-10 wt.%, calculated on the dry weight of the composition.

[0005] Next to the above additional free amino acids it is possible to use the further additional free amino acids arginine and/or glutamine which each may be present in an amount in the range of 0.1-20 wt.% calculated on the dry weight of the composition.

[0006] The peptide material is derived from wheat proteins. The protein raw material is wheat gluten protein or a subfraction thereof such as gliadin. In the present context, the term "peptide material" is understood to indicate a protein hydrolysate and may contain all types of peptides that may vary in length as well as a certain amount of free amino acids resulting from the hydrolysis. The protein raw material is hydrolysed by one or more hydrolytic enzymes. The hydrolytic enzyme can be of animal, plant, yeast, bacterial or fungal origin. Preferably enzyme preparations are used which have a low exo-peptidase activity to minimise the liberation of free amino acids and to improve taste profiles of the protein hydrolysates. The preferred hydrolysed protein material of the present invention has an average peptide chain length in the range of 2-40 amino acid residues and more preferably in the range of 3-20 amino acid residues. The average peptide chain can be determined using the method as described in WO 96/26266. The protein hydrolysates that can be used to prepare a composition as disclosed in the present invention are not limited to ones disclosed in the present invention but include all protein hydrolysates that can be obtained by enzymatic hydrolysis using common techniques as described in the literature and known to those skilled in the art. Further the peptide material is present in an amount of 0.1-50 wt.%, preferably 2-25 wt.%, calculated on dry matter basis of the composition.

[0007] The carbohydrate material component of the composition according to the invention is advantageously selected from the group consisting of mono-, di- and polysaccharides like glucose, sucrose, maltose as well as more complex edible carbohydrates such as maltodextrines. Independent on the type of carbohydrate material it is present in an amount in the range of 10-90 wt.%, preferably 50-80 wt.%, calculated on dry matter basis of the composition.

[0008] Other optional components of the composition according to the invention are vitamins, minerals, flavours, antioxidants, components having co-enzyme and antioxidant properties, lipids including emulsifiers, and proteins for meeting specific nutritional and/or physiological needs.

[0009] The composition according to the invention may have the form of a powder, a beverage or any other food

product. A beverage according to the invention can be prepared by dissolving the above-defined ingredients in an appropriate amount of water. Preferably an isotonic drink has been prepared. For drinks, intended to be used during and after exercise it is recommended to have a concentration of the composition according to the invention in the range of 10-15 wt.% calculated on the total weight of the drink.

[0010] In view of the complexicity of the processes dealing with the recovery of athletes after (exhaustive) exercise the following is remarked.

[0011] Athletes undergoing intense, prolonged training or participating in endurance races (e.g. the marathon) easily catch a cold or flu. This is most probably related to the significant decreased plasma levels of the amino acid glutamine seen during recovery after exercise at exhaustion. A marked increase in numbers of white blood cells occurred immediately after exhaustive exercise, followed by a decrease in the numbers of lymphocytes. The amino acid glutamine is essential for the optimal functioning of a number of tissues in the body, particularly of the immune system and the gut. The provision of oral glutamine after exercise appeared to have a beneficial effect on the level of subsequent infections. In addition, the activity of T-lymphocytes appeared to be increased in samples from those who received glutamine compared with placebo. If recovery between exercise bouts is inadequate, the acute effects of exercise on plasma glutamine level may be cumulative, since overload training has been shown to result in low plasma glutamine levels requiring prolonged recovery. Plasma glutamine level may be useful as an indicator of an overtrained state. As free glutamine is not stable in solution during pasteurisation and during storage, glutamine-containing peptides are the preferred glutamine source. Glutamine containing peptides can be obtained from the hydrolysis of vegetable and animal proteins, a preferred protein source is wheat gluten since this is rich in glutamine. Infections also are an important cause of morbidity and mortality in patients with multiple trauma. Studies in both animals and human beings have suggested that glutamine-enriched nutrition decreases the number of infections. In patients with multiple trauma receiving glutamine-supplemented enteral nutrition a low frequency of pneumonia, sepsis, and bacteraemia was seen.

[0012] In addition to its direct action on the cells of the immune system, glutamine may indirectly influence the immune system by the preservation of action of the antioxidant glutathione. The tripeptide glutathione (GSH) is the major intracellular antioxidant and is essential to normal cell function and replication. Studies over the last decade have demonstrated that glutamine becomes essential during metabolic stress to replete tissue GSH levels which have become depleted. The availability of glutamine appears to be important for the regeneration of GSH stores. Due to the high intake of oxygen during physical exercise there is an increased production of radicals and other forms of reactive oxygen species (ROS) in the muscle. ROS has been implicated as an underlying cause in exercise-induced disturbances in muscle homeostasis (e.g. redox status), that could result in muscle fatigue or injury. Important nonenzymatic antioxidant defences include GSH, vitamin E, vitamin C, α-lipoic acid, carotenoids, polyphenols including flavonoids and isoflavones, uric acid, bilirubin, and ubiquinone. α-Lipoic acid functions as a cofactor for α-dehydrogenase complexes and participates in the oxidative decarboxylation of α-keto acids such as pyruvate, α-ketoglutarate and branched chain α-keto acids. Normally, α-lipoic acid is present in small quantities in animal tissues and is generally bound to an enzyme complex and is therefore unavailable as an antioxidant. However, exogenous free unbound α-lipoic acid may be effective as an antioxidant and an recycling vitamin C, which increases the intracellular GSH concentration. Dietary supplementation with antioxidants has been shown to be beneficial in combating oxidative stress without enhancing performance while GSH levels were found to influence the endurance capacity of athletes.

[0013] A further aspect of the glucose uptake during and after exercise is elucidated below. In fact numerous factors determine the rate of glucose uptake during and after exercise. During exercise, one of the most important regulatory responses is an increase in blood flow to the contracting skeletal muscles. This increased blood flow provides ample substrate to the working muscles, and thus, glucose availability is usually not the rate-limiting factor for glucose utilisation. Instead, glucose transport is thought to be the rate-limiting step in glucose during exercise. Glucose transport occurs primarily by facilitated diffusion, an energy-independent process that uses GLUT-4, the major glucose carrier in human and rat skeletal muscle for transport of glucose across the plasma membrane. Both exercise and insulin increase glucose transport through an increase in the maximal velocity of transport. This increase in transport may occur through an increase in the rate that each GLUT-4 protein transport glucose (transport turnover number), an increase in the number of functional glucose transporter proteins present in the plasma membrane, or both. It appears that exercise and insulin recruit distinct GLUT-4-containing vesicles and/or mobilise different "pools" of GLUT-4 proteins in skeletal muscle originating from unique intracellular locations. The combined intake of carbohydrates and protein hydrolysates, peptides and/or amino acids will enhance the uptake of glucose during exercise by recruiting different GLUT-4 proteins to the plasma membrane of the contracting muscle cells. As a result the performance of the active muscles is enhanced and exhaustion will be delayed.

Experimental

[0014] In studies presented below the effects of intact casein, several protein hydrolysates and protein hydrolysate in combination with specific amino acids have been examined. The sodium casein used in this study is commercially

available from DMV-International. Glucose and maltodextrin were obtained from AVEBE (the Netherlands) and crystalline amino acids from BUFA (the Netherlands). In the presented study the following commercially available protein hydrolysates from Quest-International have been used: Hyprol® 3301 (whey protein hydrolysate, average peptide chain length of 4.1), Hyprol® 4107 (wheat gluten protein hydrolysate, average peptide chain length of 12.2) and Hyprol® 7102 (pea protein hydrolysate, average peptide chain length of 6.4). The insulin responses in blood plasma was analysed by radio-immuno-assay (Insulin RIA 100 kit, Pharmacia, Sweden).

[0015] In three studies the efficiency of an amino acid and/or protein (hydrolysate) mixture in a carbohydrate containing drink with respect to their insulinotropic effect in human subjects was examined. The composition of all the tested experimental drinks are given in table 1.

First study

[0016] The aim of the first study was to establish an amino acid and/or protein (hydrolysate) mixture with a maximal insulinotropic effect when co-ingested with carbohydrates.

[0017] Eight healthy, non-obese male subjects (age 21±0.4, weight 73.9±2.2 kg, height 186±2 cm, BMI 21.4±0.7 $kg \cdot m^{-2}$) after an overnight fast, were tested for 2 hours on 10 occasions in which different beverage compositions were ingested. During those trials subjects ingested 0.8 $g \cdot kg\ bw^{-1} \cdot h^{-1}$ carbohydrate and 0.4 $g \cdot kg\ bw^{-1} \cdot h^{-1}$ of an amino acid and/or protein (hydrolysate)-mixture. When the mixture of free amino acids and protein hydrolysate was tested, 0.2 $g \cdot kg\ bw^{-1} \cdot h^{-1}$ wheat gluten protein hydrolysate, 0.1 $g \cdot kg\ bw^{-1} \cdot h^{-1}$ leucine and 0.1 $g \cdot kg\ bw^{-1} \cdot h^{-1}$ phenylalanine was consumed. The drinks were ingested at a rate of 3.5 $ml \cdot kg\ bw^{-1}$ per half-hour. A strong initial increase in plasma glucose and insulin levels was observed in all trials after which large differences in insulin response between drinks became apparent. The insulin response is expressed as area under the curve during the second hour. It was found that the ingestion of the drinks containing free leucine, phenylalanine and arginine and the drinks with free leucine, phenylalanine and wheat protein hydrolysate was followed by the largest insulin response (201 and 203%, respectively; $P<0.05$) compared to the carbohydrate-only drink (see Table 1). The insulin responses correlated positively with plasma leucine, phenylalanine and tyrosine levels. The positive correlation observed with plasma tyrosine levels may be explained by the fact that the amino acid tyrosine is the hydroxylation product of phenylalanine in the liver and is formed when large amounts of phenylalanine are ingested. Ingestion of a test drink containing large amounts of free arginine (0.4 g $arginine \cdot kg\ bw^{-1} \cdot h^{-1}$) caused severe diarrhoea and the urge to defecate in all subjects for several hours during and after the trial. These gastrointestinal problems appeared to prevent intestinal absorption of the arginine as lower concentrations of arginine were seen in plasma following ingestion of other arginine containing test drinks. This indicates that in sports practice it would not be recommendable to ingest large amounts of arginine in order to stimulate growth hormone release and muscle anabolism.

[0018] The addition of glutamine to the mixture of arginine, leucine and phenylalanine had no effect on the insulin response; this suggests that, at least in the studied healthy men, *in vivo* enough glutamine is present, at least in the studied healthy men (600-800 $\mu mol \cdot 1^{-1}$ in plasma). Also the addition of free glutamine hardly influenced plasma glutamine concentrations. The drink containing the wheat gluten protein hydrolysate (drink 5) gave the highest insulin response of all tested protein hydrolysates. Although no statistical significant differences were found between the insulin responses in test drinks containing whey, pea and wheat hydrolysate vs. the control carbohydrate-only trial, the mean insulin responses were 155, 125 and 181%, respectively, compared to the control trial. There were no differences in plasma leucine and phenylalanine responses between the different protein hydrolysates tested. None of the hydrolysates gave rise to gastrointestinal or other complaints. Furthermore, the insulin responses on the ingestion of the drink containing the free amino acids leucine, phenylalanine and arginine (drink 6), the drink containing the mentioned three amino acids as well as glutamine (drink 7), as well as the drink containing wheat gluten protein hydrolysate and the free amino acids leucine and phenylalanine (drink8) were the same (table 1).

[0019] The main conclusion is that oral intake of amino acids in combination with carbohydrates can result in an insulinotropic effect as large as 200% compared to the intake of carbohydrates only. Furthermore, a mixture of free leucine, phenylalanine and arginine can produce a large insulinotropic effect when ingested in combination with carbohydrates. Surprisingly, the addition of leucine and phenylalanine to a wheat gluten protein hydrolysate created a similar insulinotropic effect as the drinks containing arginine (drink 6) but without any gastrointestinal discomfort. Following the ingestion of the intact protein (drink 2) plasma amino acid responses were in general lower compared to the responses observed following ingestion of protein hydrolysates. Therefor the use of protein hydrolysates is preferred in order to stimulate insulin secretion. Another practical disadvantage of the use of an intact protein when ingested as a drink is its poor solubility in water.

Second study

[0020] In the second study the correlation between glucose and insulin responses after oral intake of the composition

of the first study (wheat gluten protein hydrolysate, free leucine, phenylalanine and carbohydrate) with respect to the post-exercise muscle glycogen synthesis was examined. This study investigated whether an increase in carbohydrate intake and/or ingestion of a protein hydrolysate/amino acid mixture in combination with carbohydrate can increase post-exercise muscle glycogen synthesis rates when compared to the ingestion of 0.8 g·kg $bw^{-1}·h^{-1}$ carbohydrate, provided at 30-min intervals. (In Appl. Physiol., 1988 64(4) 1480 it is reported that in healthy athletes a maximum glycogen resynthesis rate is obtained upon ingestion of about 0.75 g·kg $bw^{-1}·h^{-1}$).

[0021] Eight trained cyclists (age: 24.0±0.6 years, body mass: 70.0±1.0 kg, BMI: 21.4±0.6 $m·kg^{-2}$) visited the laboratory 3 times during which a control and 2 other beverage compositions were tested. The subjects were subjected to a glycogen depletion protocol in which they cycled in two-minute block periods at alternating workload of 90 and 50% of their maximum performance capacity (maximum workload (Wmax): 390±8 W, maximum heart rate: 191±3 bts min^{-1}). This was continued until the subjects were no longer able to complete the two-minutes at 90% of their maximum. Subjects were allowed to stop when pedalling speed could not be maintained at 70% of their maximum capacity. After they had stopped muscle biopsy samples were collected and subjects received a beverage every 30 min to ensure ingestion of 0.8 g·kg $bw^{-1}·h^{-1}$ carbohydrate (CHO, drink 1), 0.8 g·kg $bw^{-1}·h^{-1}$ carbohydrate + 0.4 g·kg $bw^{-1}·h^{-1}$ wheat protein hydrolysate + free leucine and phenylalanine (CHO+PRO, drink 8) or 1.2 g·kg $bw^{-1}·h^{-1}$ carbohydrate (CHO+CHO, drink 10). After 5 hours a second biopsy was taken. Plasma insulin responses in the CHO+PRO (drink 8) and CHO+CHO trial (drink 10) were increased (table 1) compared to the CHO trial (drink 1) (+88±17 and +46±18 % respectively; $P<0.05$). Muscle glycogen synthesis was increased in both treatments compared to the CHO trial (+35.4±5.1 and +44.8±6.8 vs. 16.6±7.8 μmol glycosyl units·g $dw^{-1}·h^{-1}$, respectively: $P<0.05$).

[0022] Surprisingly, the high carbohydrate drink (CHO+CHO, drink 10) stimulated the highest glycogen synthesis in skeletal muscle but the CHO+PRO (drink 8) has the highest plasma insulin response. This suggests that the amount of glucose is limiting in the drink 8, which is the same as the control (drink 1), the amount of glucose is limiting for glycogen synthesis and indicates that post exercise ingestion of 0.8 g.kg $bW^{-1}·h^{-1}$ carbohydrate is not the maximum as is generally accepted with respect to glucose absorption as is generally accepted. More glucose can be absorbed as is shown by drink 10 which provides an intake of even 1.2 g·kg $bw^{-1}·h^{-1}$ carbohydrate.

Third study

[0023] To investigate the insulinotropic effect of protein hydrolysates and leucine and phenylalanine in combination at the high carbohydrate intake of 1.2 g·kg $bw^{-1}·h^{-1}$, a third study was performed in highly trained athletes. Here, the post-exercise insulin response as well as the plasma amino acid response following the combined ingestion of carbohydrate and wheat gluten protein hydrolysate with and without the addition of free leucine and phenylalanine in trained athletes was examined. After an overnight fast, 8 male cyclists (age: 24.0±0.6 years, body mass: 70.0±1.0 kg, BMI: 21.4±0.6 m kg^{-2}) on 5 occasions were subjected to a glycogen depletion protocol. Thereafter a control drink and 2 different beverage compositions in 2 different doses were tested. After performing the glycogen depletion protocol (see 2nd study) subjects received a beverage volume of 3.5 ml·kg bw^{-1} every 30 minutes to ensure an intake of 1.2 g·kg $bW^{-1}·h^{-1}$ carbohydrate and 0, 0.2 or 0.4 g·kg $bw^{-1}·h^{-1}$ protein hydrolysate/amino acid mixture. The insulin response is expressed as area under the curve. It was found that the ingestion of the beverages containing wheat hydrolysate, free leucine and phenylalanine resulted in a substantial increase in insulin response (+52 and +107%, respectively; $P<0.05$) compared to the control (carbohydrate only) trial (table 1). A dose related effect exists as doubling the dose (0.2 to 0.4 g·kg $bw^{-1}·h^{-1}$) lead to an additional rise in insulin response ($P<0.05$).

[0024] In contrast to our first study with subjects after an overnight fast, we found no significant increase in post-exercise insulin response following the ingestion of a wheat gluten protein hydrolysate at an intake of 0.2 or 0.4 g·kg $bw^{-1}·h^{-1}$ in combination with carbohydrate at 1.2 g·kg $bw^{-1}·h^{-1}$ compared with the control (carbohydrate-only) drink. This can partly be explained by the higher carbohydrate intake (1.2 vs. 0.8 g·kg $bw^{-1}·h^{-1}$) that was applied in the third study. Furthermore, this third study was performed following intense exercise and the insulin response is likely to be reduced as muscle contraction stimulates glucose transport, largely mediated by translocation of GLUT4 from intracellular sites to the plasma membrane as discussed earlier. Surprisingly we found that a substantial increase in insulin response is seen following the ingestion of the mixtures containing wheat gluten protein hydrolysate together with free leucine and phenylalanine when compared to the control ($P<0.05$). Ingestion of 0.2 and 0.4 g·kg $bw^{-1}·h^{-1}$ of this mixture in combination with carbohydrate resulted in an additional increase in insulin response of 51.8±9.5 and 107.4±16.7%, respectively compared to the control trial ($P<0.05$).

[0025] As both glucose availability and insulin concentrations determine the rate of glucose uptake in skeletal muscle, increasing postexercise insulin levels could have practical importance for the optimisation of glycogen synthesis rates and protein metabolism in skeletal muscle.

Table 1. Composition of used test drinks and their insulinotropic effects.

[0026] The values in the table are given in gram dry product per 100 ml drink.

Test drink	1	2	3	4	5	6	7	8*	9*	10	11	12	13	14*	15*
Intact casein		5.71													
Whey protein hydrolysate			5.71												
Pea protein hydrolysate				5.71											
Wheat protein hydrolysate					5.71			2.86	2.86			2.86	5.71	1.43	286
Leucine						1.90	1.43	1.43	0.95					0.71	1.43
Phenylalanine						1.90	1.43	1.43	0.95					0.71	1.43
Arginine						1.90	1.43		0.95						
Glutamine							1.43								
Glucose	5.71	5.71	5.71	5.71	5.71	5.71	5.71	5.71	5.71	8.57	6.85	6.85	6.85	6.85	6.85
Maltodextrin	5.71	571	5.71	5.71	5.71	5.71	5.71	5.71	5.71	8.57	10.28	10.28	10.28	10.28	10.28
Sodium saccharinate	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Citric acid	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18
Cream vanilla flavour	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50

Table continued

Test drink	1	2	3	4	5	6	7	8*	9*	10	11	12	13	14*	15*
1st Study Healthy male subjects Overnight fast	Plasma insulin response (area under curve minus baseline values): mean ± SEM (mU.m]' 2hrs **														
	4.61 ± 0.68	5.10 ± 1.44	6.64 ± 1.01	5.15 ± 0.37	7.33 ± 1.19	7.24 ± 1.15	7.16 1.49	7.10 ± 0.59	6.56 ± 0.86						
	Plasma insulin response (area under curve minus baseline values): mean ± SEM (mU·m) 1·2nd hr)***														
	2.53 ± 0.37	3.30 ± 1.05	3.93 ± 0.56	3.16 ± 0.17	4.59 ± 0.86	5.08 ± 0.88	4.61 ± 0.96	5.14 ± 0.35	4.28 0.62						
2nd Study Male athletes Post exercise	Plasma insulin response (area under curve minus baseline values): mean ± SEM (mU·ml1.5hrs)														
	8.58+ 0.86							± 15.89+ 2.21		12.27 ± 1.84					
3rd Study Male athletes Post exercise	Plasma insulin response (area under curve minus baseline values): mean ± SEM (mU·ml·1·3hrs)														
											591 ± 1.02	5.82 ± 0.42	5.83 ± 0.60	8.42 ± 0.78	11.32 ± 1.00

* drinks according to the invention

** average over two hours

*** average over the second hour

[0027] In case of the dry powder version of the composition of the present invention it is preferred to use agglomerated ingredients or to agglomerate the whole composition in order to facilitate the rehydration process. The following, non-limiting examples illustrate the embodiments according to the invention.

Example 1.

[0028] The composition as shown in table 2 was chosen such that the finally obtained drink is isotonic. Hyprol® 4107 is a wheat gluten protein hydrolysate from Quest-International. One litre of drink was prepared by dissolving 177.4 gram of the powder in an appropriate amount of water. The drink was found to be good tasting and refreshing.

Table 2. Composition of tropical sports drink.

Ingredients	Powder composition g/kg	Composition of drink %
Maltodextrin	430.70	7.64
Sugar extra fine	225.12	3.99
Hyprol® 4107	140.93	2.50
Leucine	70.47	1.25
Phenylalanine	70.47	1.25
Aspartame	1.40	0.02
Citic acid powder	37.21	0.66
Orange Flavour	5.21	0.09
Tropical Flavour	6.79	0.12
Carotenoids	0.56	0.01
Clouding agent	7.44	0.13
Tricalcium phosphate	3.72	0.07
Total	1000.00	17.74

Example 2.

[0029] A composition as shown in table 3 was chosen such that the finally obtained drink is isotonic. Hyprol® 4107 is a wheat gluten protein hydrolysate from Quest-International. One litre of drink was prepared by dissolving 165 gram of the powder in an appropriate amount of water. The drink was found to be good tasting and refreshing.

Table 3. Composition of strawberry sports drink

Ingredients	Powder composition g/kg	Composition of drink %
Maltodextrin	445.18	7.39
Sugar extra fine	221.69	3.68
Hyprol® 4107	150.60	2.50
Leucine	75.30	1.25
Phenylalanine	75.30	1.25
Aspartame	1.82	0.03
Citric acid	19.88	0.33
Strawberry flavour	1.82	0.03
Caramel	1.21	0.02
Cochineal (colour)	1.82	0.03
α-Lipoic acid	3.61	0.06
Vitamin C	1.21	0.02
Isoflavones (soy)	0.60	0.01
Total	1000.0	16.60

Example 3

[0030] Stable liquid enteral feeding for use in hospital and at home, containing both protein hydrolysate and fat (5%). Hyprol® 7102 is a pea protein hydrolysate from Quest-International. Also vitamins and calcium are added. The antioxi-

dants α-lipoic acid, flavonoids, carotenoids and the vitamins E and C are included.

Table 4. Composition of liquid enteral feeding

Ingredients	Composition of drink
30% fat emulsion NN-26751	175.00 g
Maltodextrine 27057	90.00 g
Aspartame	0.20 g
Citric acid solution 50% w/w	3.20 ml
Ascorbic acid	0.20 g
Vegetable protein Hyprol® 7102. 5 Z10418	20.00 g
Leucine	10.00 g
Phenylalanine	10.00 g
Calcium lactate 5 aq.	3.90 g
Vitamin pre-mix 961 *	0.20 g
Orange Compound Coloured QL-2319	22.50 g
Tropical NN-20325	1.50 g
α-Lipoic acid	0.60 g
Flavonoids (grape seed extract)	0.20 g
Colours (E 160 a + E 160 e)	8 mg
With water up to	1000 ml
* At the given dosage, the vitamin mix contains the RDA amounts for Vitamins B_1, B_2, B_3, B_5, B_6, B_{11}, B_{12}, C, E, and H per litre final drink	

Example 4

[0031] Nougat bar centre for a sports bar. The casein hydrolysate Hyfoama® DSN and the wheat gluten protein hydrolysate Hyprol® 4107 are both products from Quest-International. The nougat bar centres makes about 40% in weight from a sports bar, 20% can be caramel and 40% of the bar exists out of a chocolate outside.

Table 5. Composition of nougat sports bar centre

Ingredient	Composition
Sugar	375 g
Glucose (35DE)	359 g
Icing sugar	11 g
Skimmed milkpowder	43 g
Hyprol® 4107	20 g
Leucine	10 g
Phenylalanine	10 g
Hyfoama ®DSN	3.2 g
Egg albumen (powder)	2.1 g
Molten fat (hydrogenated coconut or palmkernel)	43 g
Flavour (dissolved in fat)	2.6 g
α-Lipoic acid	1.5 g
Vitamin C	0.5 g
Cocoa powder	43 g
Water	76 g
Total	1000 g

Claims

1. Composition comprising carbohydrate and peptide material and an amount of at least two additional free amino acids selected from the group consisting of leucine and phenylalanine, wherein the peptide material is obtainable by hydrolysis of protein material, the peptide material is derived from wheat protein, and the additional free amino acids leucine and phenylalanine are each present in an amount in the range of 0.2-20 wt.%, calculated on dry matter basis.

2. The composition according to claim 1, wherein the additional free amino acids leucine and phenylalanine are each present in an amount in the range of 1-10 wt% calculated on dry matter basis.

3. The composition according to claim 1 or 2, wherein a further additional free amino acid, selected from the group consisting of arginine and glutamine is present.

4. The composition according to claim 3, wherein at least one of the further additional free amino acids arginine and glutamine is present in an amount in the range of 0.1-20 wt.%, calculated on dry weight basis.

5. The composition according to any preceding claim, wherein the peptide material has an average peptide chain length in the range of 2-40 amino acids, preferably 3-20 amino acids.

6. The composition according to any preceding claim, wherein the peptide material is present in an amount in the range of 0.1-50 wt.%, preferably 2-25 wt.%, calculated on dry matter basis.

7. The composition according to any preceding claim, wherein the carbohydrate material is selected from the group consisting of mono-, di-, oligosaccharide and more complex edible carbohydrates such as maltodextrins.

8. The composition according to any preceding claim, wherein the carbohydrate material is present in an amount of 10-90 wt.%, preferably 50-80 wt.%, calculated on dry matter basis.

9. The composition according to any preceding claim, wherein one or more of vitamins, flavours, minerals, components having co-enzyme and antioxidant properties, lipids including emulsifiers, and proteins are present.

10. The composition according to any preceding claim, wherein the composition has the form of an isotonic beverage or sports bar.

11. The composition according to any preceding claim for use during or after physical exercise.

12. The composition according to any of claims 1-10 for use as an enteral clinical feeding.

Patentansprüche

1. Zusammensetzung, die Folgendes umfasst: Kohlenhydrat- und Peptidmaterial und eine Menge von mindestens zwei zusätzlichen freien Aminosäuren, die aus der Gruppe ausgewählt werden, die aus Leucin und Phenylalanin besteht, wobei das Peptidmaterial durch Hydrolyse von Proteinmaterial erhalten werden kann, das Peptidmaterial von Weizenprotein abgeleitet ist und die zusätzlichen freien Aminosäuren Leucin und Phenylalanin auf Trockenmaterialbasis berechnet jeweils in einer Menge im Bereich von 0,2-20 Gew.-% vorhanden sind.

2. Zusammensetzung nach Anspruch 1, wobei die zusätzlichen freien Aminosäuren Leucin und Phenylalanin auf Trockenmaterialbasis berechnet jeweils in einer Menge im Bereich von 1-10 Gew.-% vorhanden sind.

3. Zusammensetzung nach Anspruch 1 oder 2, wobei eine weitere zusätzliche freie Aminosäure, die aus der Gruppe ausgewählt wird, die aus Arginin und Glutamin besteht, vorhanden ist.

4. Zusammensetzung nach Anspruch 3, wobei mindestens eine der weiteren zusätzlichen freien Aminosäuren Arginin und Glutamin auf Trockengewichtbasis berechnet in einer Menge im Bereich von 0,1-20 Gew.-% vorhanden ist.

5. Zusammensetzung nach einem der vorhergehenden Ansprüche, wobei das Peptidmaterial eine durchschnittliche

Peptidkettenlänge im Bereich von 2-40 Aminosäuren, vorzugsweise 3-20 Aminosäuren, aufweist.

6. Zusammensetzung nach einem der vorhergehenden Ansprüche, wobei das Peptidmaterial auf Trockenmaterialbasis berechnet in einer Menge im Bereich von 0,1-50 Gew.-%, vorzugsweise 2-25 Gew.-%, vorhanden ist.

7. Zusammensetzung nach einem der vorhergehenden Ansprüche, wobei das Kohlenhydratmaterial aus der Gruppe ausgewählt wird, die aus Folgendem besteht: Mono-, Di-, Oligosaccharid und komplexeren essbaren Kohlenhydraten, wie beispielsweise Maltodextrinen.

8. Zusammensetzung nach einem der vorhergehenden Ansprüche, wobei das Kohlenhydratmaterial auf Trockenmaterialbasis berechnet in einer Menge im Bereich von 10-90 Gew.%, vorzugsweise 50-80 Gew.-%, vorhanden ist.

9. Zusammensetzung nach einem der vorhergehenden Ansprüche, wobei Vitamine, Geschmacksstoffe, Mineralien, Komponenten mit Coenzym- und Antioxidationseigenschaften, Lipide, einschließlich Emulgatoren, und/oder Proteine vorhanden sind.

10. Zusammensetzung nach einem der vorhergehenden Ansprüche, wobei die Zusammensetzung die Form eines isotonischen Getränks oder eines Sportriegels aufweist.

11. Zusammensetzung nach einem der vorhergehenden Ansprüche zur Verwendung während oder nach einem physischen Training.

12. Zusammensetzung nach einem der Ansprüche 1-10 zur Verwendung als enterale klinische Nahrung.

Revendications

1. Composition comprenant une matière d'hydrate de carbone et une matière peptidique ainsi qu'une quantité d'au moins deux acides aminés libres supplémentaires sélectionnés dans le groupe consistant en leucine et phénylalanine,
dans laquelle la matière peptidique peut être obtenue par hydrolyse d'une matière protéinique, la matière peptidique est dérivée de la protéine de blé, et les acides aminés libres supplémentaires de leucine et phénylalanine sont chacun présents dans une quantité allant de 0,2 à 20 %, taux calculé d'après le poids de matière sèche.

2. Composition selon la revendication 1, dans laquelle les acides aminés libres supplémentaires de leucine et phénylalanine sont chacun présents dans une quantité allant de 1 à 10%, taux calculé d'après le poids de matière sèche.

3. Composition selon la revendication 1 ou 2, dans laquelle est présent un autre acide aminé libre supplémentaire du groupe consistant en arginine et glutamine.

4. Composition selon la revendication 3, dans laquelle est présent au moins un des autres acides aminés libres supplémentaires d'arginine et de glutamine dans une quantité allant de 0,1 à 20 %, taux calculé d'après le poids de matière sèche.

5. Composition selon l'une quelconque des revendications précédentes, dans laquelle la matière peptidique a une longueur de chaîne peptidique moyenne allant de 2 à 40 acides aminés, de préférence de 3 à 20 acides aminés.

6. Composition selon l'une quelconque des revendications précédentes, dans laquelle la matière peptidique est présente dans une quantité allant de 0,1 à 50 %, de préférence de 2 à 25 %, taux calculés d'après le poids de matière sèche.

7. Composition selon l'une quelconque des revendications précédentes, dans laquelle la matière d'hydrate de carbone est sélectionnée dans le groupe consistant en mono-, di-, oligo-saccharide et hydrates de carbone comestibles plus complexes tels que les maltodextrines.

8. Composition selon l'une quelconque des revendications précédentes, dans laquelle la matière d'hydrate de carbone est présente dans une quantité de 10 à 90 %, de préférence de 50 à 80 %, taux calculés d'après le poids de matière sèche.

9. Composition selon l'une quelconque des revendications précédentes, dans laquelle sont présents un ou plusieurs de vitamines, arômes, minéraux, composants ayant des propriétés de co-enzyme et d'antioxydant, lipides comportant des émulsifiants et protéines.

10. Composition selon l'une quelconque des revendications précédentes, dans laquelle la composition a la forme d'une boisson isotonique ou d'une barre alimentaire pour sportifs.

11. Composition selon l'une quelconque des revendications précédentes, destinée à être utilisée durant ou après un exercice physique.

12. Composition selon l'une quelconque des revendications 1 à 10, destinée à être utilisée comme alimentation clinique entérale.